GENETIC ORGANIZATION AND ADAPTIVE RESPONSE OF ALLOZYMES TO ECOLOGICAL VARIABLES IN *FUNDULUS HETEROCLITUS*[1,2]

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ABSTRACT

Populations of *Fundulus heteroclitus*, (Cyprinodontidae) a widespread coastal marine fish, were studied in control and artificially heated environments on the North Shore of Long Island, New York to determine (1) patterns of variation in biochemical phenotypes and (2) the extent to which this variation reflected adaptation to environmental characteristics.——Variation at three of twelve polymorphic isoenzyme loci from the warm water population was beyond the range of variation among control populations, and resembled those determined for populations living at more southern latitudes. Hence, these differences were interpreted as adaptations to warm environments. Significant differences in allele frequencies and zygotic proportions at ten of twelve isoenzyme loci were found associated with differences in environments, sexes, and/or age classes. These data strongly support the view that protein polymorphisms are adaptive.——Several observations suggested that selection acts upon multilocus phenotypes rather than upon those of single loci. Several di-locus phenotypic distributions were demonstrated to be nonrandom, and those that exhibited similar patterns of dependence over years were postulated to be maintained by selection. Highly heterozygous fish exhibited superior viability when cohorts were compared over successive years.——The consequences of the polygynous mating system in this species for maintaining genetic variation and for allowing rapid evolutionary response to a variable environment are discussed.

THIS study is an attempt to determine the adaptive significance of protein polymorphisms in natural populations of a coastal marine fish, *Fundulus heteroclitus*. Evidence for the adaptive significance of protein polymorphisms is sought in tests involving ecological conditions, demography, and the distribution of non-homologous alleles.

Many studies have analyzed the genetic response of populations, both in population cages and in natural environments, to ecological variables. Clinal variation in gene frequencies, often correlated with temperature, has been discovered in a number of studies (KOEHN and RASMUSSEN 1967; MERRITT 1972; WILLIAMS,

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KOEHN and MITTON 1973). Kinetic analyses of allelic proteins support the adaptive nature of the variation in some of these studies (KOEHN 1969a; MERRITT 1972), but many observations are open to alternative interpretations (OHTA and KIMURA 1971; KIMRUA and OHTA 1971). More complex relationships between genetic variation and environmental heterogeneity have been illuminated with multivariate analyses (JOHNSON *et al.* 1969; TAYLOR and MITTON 1974). The major environmental component analyzed here is temperature. Parallel variation in genetic response to an industrial thermal effluent and a natural latitudinal gradient in temperature is a test of adaptive response of protein polymorphisms to temperature.

Evidence of the adaptive nature of protein polymorphisms from demographic comparisons is not plentiful. Reported studies, however, are in agreement in the observation of superior viability in heterozygous individuals (FUJINO and KANG 1968; TINKLE and Selander 1973; KOEHN, TURANO and MITTON 1973; CLEGG and ALLARD 1973). A multi-locus estimate of heterozygosity, based on twelve polymorphic protein loci, is monitored here as a function of age among individuals of a cohort.

Theoretical studies (LEWONTIN 1964; FRANKLIN and LEWONTIN 1970) suggest that stable linkage disequilibrium may result in natural populations if limited combinations of linkage and fitness interactions exist between loci. Apparent linkage disequilibrium may be produced by sampling error (SVED 1968a) or gene flow (MITTON, KOEHN and PROUT 1973), but otherwise might be interpreted as evidence of selection upon the loci involved (CLEGG, ALLARD and KAHLER 1972; ALLARD *et al.* 1972). Evidence for the adaptive role of protein polymorphisms is sought in the joint distributions of loci, expressed as linkage disequilibrium.

In addition to the exploration of the role of protein markers, this study is an analysis of adaptation and evolution in fluctuating environments. Mating system, sex ratio, and intensities of selection are considered in an effort to integrate models in population genetics and observations taken from the field.

MATERIALS AND METHODS

Sampling Design

Fish were collected from nine localities on the North Shore of Long Island, New York, in the fall of 1971 and/or 1972. The localities, in order from west to east, are Huntington Harbor, Centerport, Asharoken, Northport, the Nissequogue River, West Meadow Creek, Flax Pond, Setauket Harbor, and Mt. Sinai Harbor (Figure 1). All localities except Northport represent natural habitats (bays, estuaries, salt marshes) for this species. Northport represents a novel environment, since fish collected there were taken from a cooling pond of an electric generating plant that uses water from Long Island Sound to cool condenser tubes. *F. heteroclitus* resident at the power plant experience temperatures 14 to 16 degrees Centrigrade above those of surrounding localities.

An additional sample of fish to be used for comparison of allozyme frequencies was collected in 1971 in Mystic Isle, New Jersey, approximately 100 miles south of Long Island Sound. Temperature data show Long Island Sound and southern New Jersey to have similar mean surface water temperatures, but the maximum temperatures recorded in New Jersey are 3 to 4 degrees Centrigrade warmer than those observed in Long Island Sound.

LONG ISLAND SOUND

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Figure shows a map of Long Island Sound with sample localities numbered 1-9.

1 HUNTINGTON HARBOR
2 CENTERPORT
3 ASHAROKEN
4 NORTHPORT
5 NISSEQUOGUE RIVER
6 WEST MEADOW CREEK
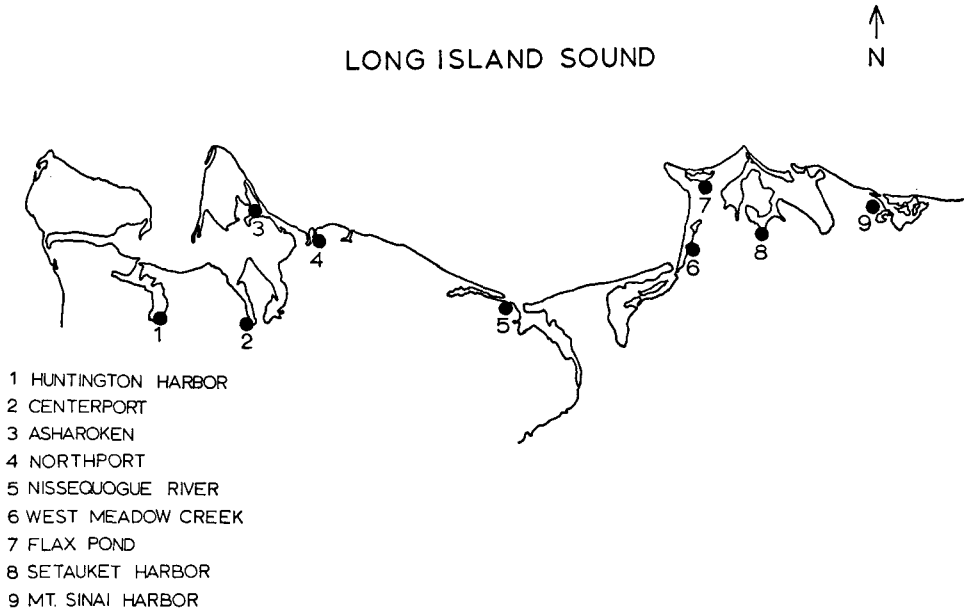7 FLAX POND
8 SETAUKET HARBOR
9 MT. SINAI HARBOR

FIGURE 1.—Sample localities on the North Shore of Long Island, N.Y.

Fish were collected in traps, cylindrical wire cages with indented, conical openings at each end, baited with crushed mussels.

Experimental Subject

Fundulus heteroclitus, a euryhaline cyprinodontid fish known commonly as the mummichog or killifish, is found commonly in bays, estuaries, and salt marshes from Labrador to the Matanzas River in Florida. *F. heteroclitus* spawn in shallow water from April to August, and become sexually mature in one year. The fish pass much of the winter burrowed in the mud, and some fish survive a second winter. They exhibit striking sexual dimorphism, especially when spawning.

Electrophoretic Methods

Serum samples were taken from live fish and subjected to horizontal starch gel electrophoresis as previously described (KOEHN and RASMUSSEN 1967), employing the discontinuous lithium hydroxide buffer of SELANDER, HUNT and YANG (1969). Serum proteins, including one polymorphic protein (SERP), were stained with Amido Black 10B and bands were visible after removal of excess stain by immersion in a solution of water, acetic acid, and methanol (5:1:5) for several hours. Serum esterase (SERE) was demonstrated by staining in a solution of Fast Blue RR diazonium salt and 1% α-napthyl butyrate at pH 7.0.

The majority of proteins studied were from the liver. Livers were excised from thawed fish, placed in one volume of 0.1 M Tris-HCl pH 7.0 buffer, sonicated for 1 to 5 seconds, centrifuged at 1800 g for 3 minutes, and stored at —60° until time of electrophoresis.

The following detection techniques for enzymes were modified from other authors. An aqueous solution prepared as described by an author was mixed with an equal volume of molten agar cooled to 60°, poured over the sliced gel, and allowed to cool. Tetrazolium or diazonium precipitates were easily visible through the agar.

Lactate dehydrogenase [LDH; E.C. 1.1.1.25.] and malate dehydrogenase [MDH; E.C. 1.1.1.37.] were resolved as in WHITT (1970a) and visualized according to WHITT (1970a,b).

All other enzymes were resolved in starch gel utilizing the lithium hydroxide buffer of SELANDER, HUNT and YANG (1969). In addition to the serum esterase, four esterase loci (EST-1, EST-2, EST-3, EST-4) could be detected in liver homogenates. Phosphohexose isomerase [PHI; E.C. 5.3.1.9.], glucose-6-phosphate dehydrogenase [G6PD; E.C. 1.1.1.49.], and two phosphoglucomutase loci [PGM-1, PGM-2; E.C. 2.7.5.1.] were visualized by the methods of SHAW and PRASAD (1970).

Hemoglobin (HB-1, HB-2) was prepared by the method of KOEHN (1969b), and resolved on the lithium hydroxide buffer. The lactate dehydrogenase most active in eye and muscle were prepared from homogenates from those tissues and resolved and visualized in the same manner as the heart LDH locus. Tetrazolium oxidase [TO] was a byproduct of any of the dehydrogenase stains, if they were allowed to incubate for a sufficient amount of time. Glucokinase [GK; E.C. 2.7.1.2.], xanthine dehydrogenase [XDH; E.C. 1.2.1.3.], glutamate dehydrogenase [GDH; E.C. 1.4.1.2.], 6-phosphogluconate dehydrogenase [6PDH; E.C. 1.1.1.43.], glycerol-3-phosphate dehydrogenase [GPDH; E.C. 1.1.1.8.], acid phosphotase [ACP; E.C. 3.1.3.2.], and aminopeptidase [AP; E.C. 3.4.1.2.] resolved on the lithium hydroxide buffer, and were demonstrated by the methods of SHAW and PRASAD (1970).

Nomenclature of allelic products corresponds to their relative mobility in the specified buffer system. The fastest migrating proteins were designated a, followed by b, c, etc. Phenotypes are described by listing the different allelic products present. Rare alleles (frequency less than .01) were pooled with those alleles most similar in electrophoretic mobility.

Breeding experiments have confirmed the mendelian inheritance of LDH and MDH (WHITT 1970a,b). Support for the hypothesis that other proteins in this study have mendelian inheritance comes from the observation of all the expected phenotypes, in patterns observed in other species for which breeding data are complete.

Statistical Methods

Age classes were determined by the method of BHATTACHARYA (1967) to resolve Gaussian components from a frequency distribution.

Homogeneity of allele frequencies among localities and year classes was tested with a weighted chi-square test (WORKMAN and NISWANDER 1970).

Estimates of linkage disequilibrium (LEWONTIN and KOJIMA 1960) or gametic phase unbalance (JAIN and ALLARD 1966) were taken from zygotic matrices by the maximum likelihood solution of BENNETT (1965) and HILL (1974). For comparison with results of other studies, D' (LEWONTIN 1964) and r (WRIGHT 1933) are also presented.

RESULTS

Twenty-five loci sampled from 65 individuals captured at Flax Pond in 1971 were utilized to estimate the percentage of loci polymorphic, and the percentage of heterozygous loci per individual. In this sample, 56% of the loci studied were polymorphic, and individuals were heterozygous at an average of 18% of the loci. Therefore, this species exhibits substantial genetic variability, in comparison to both vertebrate species (AVISE and SELANDER 1972) and species of Drosophila (RICHMOND 1972).

Variation at 12 polymorphic protein-encoding loci sampled in the heated area was tested for variation beyond the range seen in the eight control localities. Variation hypothesized to be a direct response to temperature may be subjected to further testing on a latitudinally-based temperature gradient. Chi-square tests of homogeneity are presented in Table 1 for control population samples and for all samples from the North Shore of Long Island. These test a combination of geographic and annual homogeneity in the control samples and of this plus

TABLE 1

Tests of homogeneity on allele frequencies for 12 polymorphic proteins in F. heteroclitus

Proteins	χ^2	All samples df	P	χ^2	Control samples df	P
SERP	14.89	7	<.050	13.92	5	<.025
SERE	20.83	10	<.025	17.59	8	<.025
EST-1	204.09	9	<.001	120.62	7	<.001
EST-2-a	86.58	10	<.001	47.51	8	<.001
EST-2-b	102.80	10	<.001	42.22	8	<.001
EST-3	61.74	10	<.001	36.54	8	<.001
EST-4	44.74	10	<.001	13.35	8	>.100
LDH	18.64	10	<.050	8.35	8	>.500
MDH	396.12	10	<.001	29.70	8	<.005
PHI-a	65.14	10	<.001	48.00	8	<.001
PHI-b	45.19	10	<.001	45.47	8	<.001
G6PD-a	26.30	10	<.005	23.61	8	<.005
G6PD-b	16.86	10	>.050	15.41	8	>.050
PGM-1-a	11.95	8	>.100	7.28	6	>.100
PGM-1-b	17.99	8	<.025	14.29	6	<.050
PGM-2	3.78	9	>.900	2.13	7	>.900

Explanation: Tests have been performed on the faster allele of diallelic polymorphisms, and upon the two fastest alleles of triallelic polymorphisms.

environmental homogeneity in all samples. Heterogeneity at the majority of loci studied may indicate populations adapted to natural but slightly different environments, or local stochastic differentiation in populations connected by little migration. Inclusion of allele frequency estimates from Northport collections makes the test of homogeneity reject the null hypothesis for EST-4 and LDH, suggesting a response of allele frequencies to some component of the environment at Northport.

Temporal and geographic variation at the MDH locus is presented in Table 2. The frequency of MDH-a varies from 1.0 to .913 in samples from control localities. At Northport, however, the average frequency is .73, which is beyond the normal range of variation of this allele in Long Island Sound.

The hypothesis that adaptive responses at Northport include alleles introducing heterogeneity at LDH, EST-4, and an increase of MDH-b, can be tested by sampling these gene frequencies in a warmer natural environment. Alleles that endow their carriers with greater fitness at higher temperatures should be found in higher frequencies in warmer environments.

Support for the hypothesis of adaptive response to temperature for LDH, EST-4, and MDH is found in allele frequency estimates for these polymorphisms taken from fish captured at Mystic Isle, N.J. MDH-b, which is more common at Northport (Table 2) than surrounding localities, is almost fixed in southern New Jersey. LDH-a is responsible for the heterogeneity of allele frequencies at Northport and is higher in frequency in New Jersey than any of the localities in Long Island Sound. There is good agreement in the frequencies of the slow EST-4 allele at Northport and Mystic Isle, N.J.

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TABLE 2

Allelic frequencies and zygotic distributions of MDH in 12 population samples of F. heteroclitus

Samples	aa	ab	bb	n	p(a) ± SE	χ²
Huntington Harbor	48.0	0.0	0.0	48	1.000 ± .000	.00
	48.0	0.0	0.0			
Centerport	45.0	3.0	0.0	48	.969 ± .018	.05
	45.0	2.9	.1			
Asharoken	154.0	23.0	3.0	180	.919 ± .014	3.40
	152.2	26.7	1.1			
Northport 1971	173.0	128.0	28.0	329	.720 ± .017	.39
	170.7	132.6	25.7			
Northport 1972	174.0	144.0	15.0	333	.739 ± .017	4.82
	181.7	128.5	22.8			
Nissequogue	40.0	8.0	0.0	48	.917 ± .028	.40
	40.3	7.3	.4			
West Meadow	50.0	2.0	0.0	52	.981 ± .013	.00
	50.0	2.0	0.0			
Flax Pond 1971	291.0	17.0	0.0	308	.972 ± .007	.25
	291.2	16.5	.3			
Flax Pond 1972	259.0	26.0	1.0	286	.951 ± .009	.16
	258.7	26.6	.7			
Setauket Harbor	44.0	7.0	1.0	52	.913 ± .028	1.14
	43.4	8.2	.4			
Mt. Sinai Harbor	47.0	0.0	0.0	47	1.000 ± .000	.00
	47.0	0.0	0.0			
Mystic Isle, N.J.	0.0	2.0	50.0	52	.019 ± .013	.02
	0.0	2.0	50.0			

Explanation: From left to right the following information is given in the columns of this table: observed zygotic frequencies (above) and expected frequencies (below), sample sizes (n), allelic frequencies [p(a)] and their standard errors (SE), and χ² from tests of goodness of fit of observations to Hardy-Weinberg expectations.

Adaptive significance of a protein polymorphism may also be inferred from different allele frequencies or zygotic proportions between sexes. In the absence of sex-limited inheritance, the separate sexes should have the same gene frequencies and zygotic proportions, if unaltered by selection. Collections with adequate sample sizes were tested for sex-dependent differences. Occasional significant differences were found in these comparisons, but only those that were consistent over years will be discussed. SERE, the esterase predominant in serum and muscle, showed consistent and significant sex differences in gene frequencies at Flax Pond in 1971 and 1972, while the fit to Hardy-Weinberg expectations was good for each sex (Table 3). At Northport, allele frequencies did not differ between males and females, but there was a difference with respect to zygotic proportions. Males in both years sampled corresponded to Hardy-Weinberg expectations, but females showed poor fits, in each case due to a deficiency of heterozygotes. At the EST-1 locus, males had significantly lower frequencies of the faster allele, and this difference was consistent with respect to both years and environments (Table 4). Males had lower frequencies of the faster allele at the

TABLE 3

Allelic frequencies and zygotic distributions of SERE for males and females in F. heteroclitus

Samples	Sex	aa	ab	bb	n	p(a) ± SE	χ^2
Flax Pond 1971	♂	64	40	9	113	.743 ± .029	.59
	♀	127	58	9	194	.804 ± .020	.50
Flax Pond 1972	♂	83	62	11	156	.731 ± .025	.02
	♀	89	51	5	145	.790 ± .024	.50
Northport 1971	♂	78	37	6	119	.803 ± .026	.63
	♀	130	55	18	214	.787 ± .020	11.56
Northport 1972	♂	91	51	6	148	.787 ± .024	.12
	♀	109	60	16	185	.751 ± .022	3.22

Explanation: From left to right the following information is given in the columns of this table: observed zygotic frequencies, sample sizes (n), allelic frequencies [p(a)] and their standard errors (SE), and χ^2 from tests of goodness of fit of observations to Hardy-Weinberg expectations.

TABLE 4

Allelic frequencies and zygotic distributions of EST-1 for males and females in F. heteroclitus

Samples	Sex	aa	ab	bb	n	p(a) ± SE	χ^2
Flax Pond 1971	♂	6	19	20	45	.344 ± .050	.19
	♀	17	48	31	96	.427 ± .036	.04
Flax Pond 1972	♂	7	58	90	155	.232 ± .024	.38
	♀	9	71	68	148	.301 ± .027	2.93
Northport 1971	♂	15	32	74	121	.256 ± .028	11.33
	♀	32	76	106	215	.326 ± .023	8.18
Northport 1972	♂	0	9	108	117	.038 ± .013	.01
	♀	3	39	123	165	.136 ± .019	.00

For explanation see note below Table 3.

TABLE 5

Allelic frequencies and zygotic distributions of EST-3 for males and females in F. heteroclitus

Samples	Sex	aa	ab	bb	n	p(a) ± SE	χ^2
Flax Pond 1971	♂	73	31	8	112	.790 ± .027	3.06
	♀	154	35	7	196	.875 ± .017	6.61
Flax Pond 1972	♂	81	44	21	147	.701 ± .026	11.11
	♀	95	36	5	143	.808 ± .022	.08
Northport 1971	♂	97	20	3	120	.892 ± .020	2.26
	♀	169	33	11	214	.871 ± .016	20.77
Northport 1972	♂	97	37	11	148	.780 ± .023	6.76
	♀	118	44	23	185	.757 ± .022	23.18

For explanation see note below Table 3.

TABLE 6

Allelic frequencies and zygotic distributions of MDH for males and females in F. heteroclitus

Samples	Sex	aa	ab	bb	n	p(a) $\pm$ SE	χ^2
Flax Pond 1971	♂	109	4	0	113	.982 ± .009	.00
	♀	182	13	0	196	.962 ± .009	.02
Flax Pond 1972	♂	146	12	0	158	.962 ± .011	.02
	♀	133	14	1	148	.946 ± .013	.09
Northport 1971	♂	55	51	12	120	.671 ± .030	.04
	♀	117	77	16	212	.738 ± .021	.48
Northport 1972	♂	69	72	7	148	.709 ± .026	4.80
	♀	105	72	8	185	.762 ± .022	.99

For explanation see note below Table 3.

EST-3 locus (Table 5) at Flax Pond, but allele frequencies were not significantly different at Northport. As with the SERE locus, females at Northport had a considerable heterozygote deficiency at the EST-3 locus which may or may not be found in males. MDH, which has been shown to have different allele frequencies in different environments (Table 2), also exhibited differences with sex (Table 6). For both 1971 and 1972, the frequency of the fast allele was higher in males at Flax Pond, but higher in females at Northport. Although no insight concerning the mode of selection can be gained from these observations, consistent differences with respect to sex do suggest an adaptive role for these polymorphisms.

Allelic frequencies that change from age class to age class might indicate selection of two different types. A monotonic progression in gene frequency on year class might indicate that a particular allele was related to viability with respect to a constant environmental stress factor. Significant non-monotonic frequency changes on year class that did not repeat themselves regularly might relate to chance biological or environmental events (such as migration or climatic fluctuations).

A frequency distribution of total lengths of fishes was resolved by the method of BHATTACHARYA (1967) into Gaussian components that may be interpreted as age classes. Three age classes were present in all samples, and total lengths approximating cut-off points between year classes were 70mm and 90mm. For each year class of the largest samples, the sex ratios (% male) and sample sizes are given in Table 7. Samples from Flax Pond and Northport were separated into year classes, and tested for heterogeneity. Seven of the fifty-six comparisons, involving EST-1, EST-2, EST-3, PHI, and PGM-1, were significantly heterogeneous, which is more than the two or three expected by chance alone. However, patterns of heterogeneity suggestive of a change correlated with year classes did not repeat themselves. Thus, selective forces acting either upon larval stages and juveniles or upon spawning adults possibly differ from year to year.

Some models in population genetic theory accommodate high amounts of genetic variability with low amounts of genetic load (MILKMAN 1967; SVED,

TABLE 7

Sex ratios (% male) and sample sizes (n) for year classes in F. heteroclitus

	One		Year classes Two		Three	
Collection	%	n	%	n	%	n
Asharoken 1971	64	25	38	110	18	45
Flax Pond 1971	57	68	32	209	18	33
Flax Pond 1972	77	79	46	175	33	52
Northport 1971	49	69	35	232	9	32
Northport 1972	58	52	45	244	21	42

REED and BODMER 1967; KING 1967; WILLS, CRENSHAW and VITALE 1970; FRANKLIN and LEWONTIN 1970) and are consistent with the premise that highly heterozygous individuals will be endowed with higher fitness than the mean. Collections with large sample sizes were used to test this premise.

To determine if heterozygosity increased with year class, the heterozygosity of a cohort may be tested directly. The number of loci observed to be heterozygous was divided by the total number of loci successfully typed in that individual, and a standard error was calculated for each age class in each sample. A cohort in the first year class will be the same as the second year class the following year, transformed only by differential survival during the intervening winter. Viewed in this way, there was good agreement at both Flax Pond and Northport to the theoretical expectations of greater viability in highly heterozygous individuals (Figure 2). At both localities, a significant increase in heterozygosity of a cohort occurred for cohorts starting the first year class.

To characterize the level of genetic organization within the set of protein markers, estimates of D and D', the linkage disequilibrium or gametic phase unbalance parameters, and r, the correlation between loci, were calculated from zygotic matrices for all pairs of the 12 allozyme loci. Only one of the 66 pairs of loci, SERE and EST-3, gave consistently significant results over both environments and years (Table 8).

Both loci involved in this interaction have different frequencies in the sexes (Tables 3 and 5), which suggests this interaction may be partially due to sex-dependent modes or intensities of selection. A dependency in distribution of phenotypes could be due to selection acting upon single loci, if selection results in different gene frequencies in sexes (MITTON, KOEHN and PROUT 1973), simulating a Wahlund effect, in sexes rather than populations. The magnitude of D and D', however, as well as tests upon the sexes separately (Table 8), show that this association is a product of selection acting jointly upon the two loci in both sexes.

DISCUSSION

F. heteroclitus spawned in the thermal effluent at Northport, New York find themselves in an odd predicament. Their parents have passed to them a set of genes that adapts the majority of individuals to the climate of Long Island Sound.
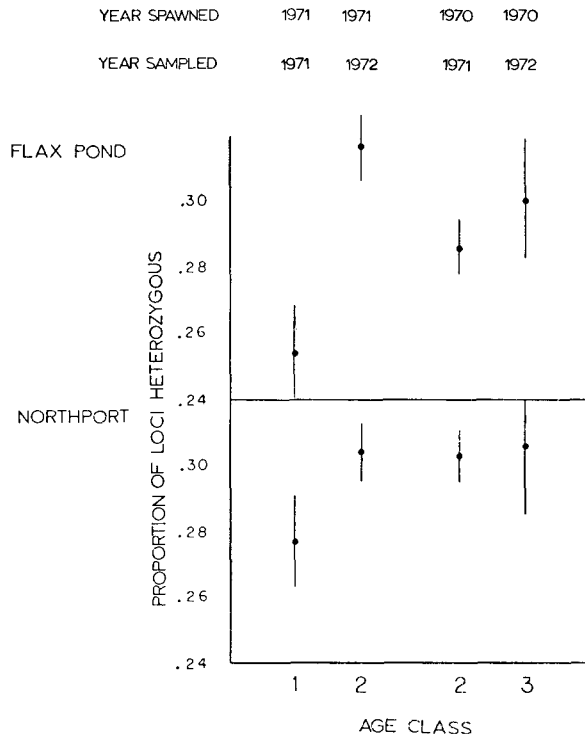
FIGURE 2.—Observed levels of heterozygosity ($Y \pm SE$) of age classes of *F. heteroclitus* captured at two localities in two years, based upon 12 polymorphic loci.

TABLE 8

Estimates of D, D', and r for SERE and EST-3 in 4 Long Island population samples

Population samples		D	D'	r	N	χ^2
Flax Pond 1971	Whole sample	.066	.538	.438	306	58.8
	♀ ♀	.090	.582	.502	112	28.2
	♂ ♂	.052	.512	.395	194	30.2
Flax Pond 1972	Whole sample	.075	.420	.416	282	48.2
	♀ ♀	.076	.394	.390	141	21.4
	♂ ♂	.092	.731	.594	141	46.3
Northport 1971	Whole sample	.072	.744	.545	331	98.3
	♀ ♀	.060	.718	.504	116	29.5
	♂ ♂	.075	.747	.554	218	65.7
Northport 1972	Whole sample	.096	.548	.543	334	98.6
	♀ ♀	.050	.310	.307	146	13.8
	♂ ♂	.127	.706	.690	188	89.6

NOTE: All tests have 1 degree of freedom. $\chi^2[1, .01] = 6.64$.

The environment, however, is more similar to, and even warmer than natural localities a thousand miles to the south. For instance, the mean surface water temperature is about 12° for Long Island Sound, and may be as great as 25° at Northport. Some individuals will have been endowed with a genetic constitution incapable of operating their metabolic machinery under prevailing conditions, while others, through segregation and recombination, will possess either the right genes or the right assortment of genes to carry on normal activities. A combination of characters or genes in the latter have been favored by selection.

Direct evidence that the heated effluent at Northport imposes a stress on *F. heteroclitus* is found in a study of morphological variables (MITTON 1973). High incidence of vertebral abnormalities, as well as modification of the head morphology, document the development stress and selection for a phenotype resembling that of more southern latitudes.

For most of the twelve protein polymorphisms in this study there is some indication that this selection influences their gene frequencies or zygotic proportions. Differences have been found between sexes (Tables 3, 4, 5, and 6) and among year classes. Two loci give consistent evidence of linkage disequilibrium (Table 8). Three of the twelve protein loci show some response to the novel thermal environment that suggests a direct response to temperature. This study, therefore, supports the hypothesis that protein polymorphisms play an active role in adaptation, and are subject to laws and principles subsumed under Neo-Darwinian evolutionary theory.

F. heteroclitus is a polygynous animal, and shows marked sexual dimorphism (NEWMAN 1907). Males court females, and fight among themselves during the spawning season (NEWMAN 1907). The breakdown of the year classes by sex also suggests strong differences in viability (Table 7), for sex ratio (% male) decreases with age in both comparisons among and within cohorts. Some of the differences among age classes may be confounded by different growth rates or schedules in the sexes, but the extent to which this is a bias is unknown.

GIESEL (1972) has considered the consequences of a polygynous mating system for the rate of evolution and efficiency of a population to effectively track a changing or variable environment. The potential rate of gene frequency change is directly proportional to the degree to which male selective mortality exceeds female selective mortality. The shifting proportion of sexes in the year classes of *F. heteroclitus* (Table 7) and accounts of mating behavior (NEWMAN 1907) suggest that the arguments put forward by GIESEL (1972) would apply to *F. heteroclitus*. If the continuing mortality in males in the third year is selective, the few large males left will be the best adapted to that particular environment, and will have a large number of females to mate with. Therefore, the alleles present in the few largest, best adapted males will have a large representation in the following generation, enabling a population to alter gene frequencies and zygotic proportions in such a way that the adaptation of the population is maintained at a high level, even when the environment is changing in an unpredictable way.

Direct evidence of differential viability in the sexes comes from the analysis of gene frequency differences between the sexes (Tables 3, 4, 5, 6). Males had different gene frequencies than females at four of the twelve loci monitored, indicating different viabilities in the sexes. Thus the hypothesis that a greater selective mortality in males and a polygynous mating system may allow a population to more efficiently track its environment (GIESEL 1972) may in part explain the heterogeneity of gene frequencies seen in the different year classes of *F. heteroclitus*.

When a cohort was analyzed over successive years, the proportion of polymorphic loci increased significantly at both Flax Pond and Northport for the first year class, and perhaps subtly for the second year class. This diminution of increase in heterozygosity suggests that the first over-wintering period removes those not physiologically capable of hibernation, leaving little opportunity for improvement the second year. It should be pointed out that only individuals that have survived at least one winter are capable of reproduction. Because individuals of the first age class are not capable of reproducing, an advantage in viability necessarily confers an advantage in reproduction. These results suggest that reproducing individuals are significantly more heterozygous than the cohort they were selected from.

Results reported here may shed some light on the maintenance of high levels of variability in natural populations. The data collected for this study, when analyzed at the single-gene level, give no evidence of overdominance. In fact, consistent heterozygote deficiencies were found at the EST-2, EST-3, G6PD and PGM-1 loci. Yet when a cohort is sampled in consecutive years, highly heterozygous individuals exhibit greater viability. Experimental evidence has demonstrated a substantial advantage for heterozygous individuals in several species of Drosophila (SVED 1968b; SVED and AYALA 1970; MOURAO, AYALA and ANDERSON 1972; TRACEY and AYALA 1974) and plants (CLEGG and ALLARD 1973), yet the evidence of single-gene heterosis has not been compelling (but see KOEHN 1969a, WILLS and NICHOLS 1971; and SING, BREWER and THIRTLE 1973). This apparent contradiction of data must be due to the fact that individual genes are imbedded in chromosomes with many other genes, and the performance of any allele, when viewed in the context of a natural population, will be compromised by proximal loci (WILLS and NICHOLS 1972). That is, the performance of any gene will depend upon its genetic background, and particularly upon closely linked genes. Favorable combinations of genes may accumulate in frequency under selection (FRANKLIN and LEWONTIN 1970), and linger for generations, even in the absence of selection (SVED 1971).

The impression of the genetics of a natural population derived from this study is one of a dynamic state of flux, in which the polygynous mating system and separate viabilities and fecundities of the sexes operate to keep the population adapted to an inconstant environment. While separate gene loci adjust gene frequencies or zygotic proportions to the demands of the separate sexual strategies or to the whims of the environment, there appears to be a premium on heterozygosity *per se*. Loci coding for structural proteins and loci determining or con-

tributing to the states of morphological variables are found in combinations not expected under assumptions of independence, an observation in agreement with the latest theory in population genetics.

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