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Population Genetics of *Tigriopus californicus* (Copepoda: Harpacticoida): I. Population Structure Along the Central California Coast*

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ABSTRACT: Polyacrylamide gel electrophoresis revealed three genetic polymorphisms among central California populations of the supralittoral copepod *Tigriopus californicus*. Laboratory analysis of mated pairs and their progeny confirmed the allelic nature of esterase (EST), phosphoglucose isomerase (PGI), and leucine aminopeptidase (LAP) electromorphs. Polymorphism within each enzyme system was localized such that only a few of the nine or more sites sampled were polymorphic while the others were fixed on the same allele. While the Pescadero site (located near the middle of the 250 km transect studied) was polymorphic for PGI with two alleles at 0.5 frequency, one of these forms never reached a frequency of 0.04 or higher in any of the other populations sampled, including a population located only 1.5 km to the south. Similar population differentiation was observed with respect to the EST locus, and to a lesser extent, the LAP locus. EST phenotype frequencies at Moss Beach exhibited both microgeographic and temporal variation. No obvious patterns, however, were observed among the phenotype frequencies, and estimated allele frequencies indicate that remarkable consistency existed among all the Moss Beach population samples. These data indicate that *T. californicus* populations located within habitat patches are genetically relatively homogeneous, while populations occupying habitats isolated by stretches of sandy beach can show strong genetic differentiation over short geographic distances.

INTRODUCTION

The supralittoral zone is probably unique among marine habitats in that it is characterized by extreme environmental fluctuations. Tidepools within this zone are never submerged by the tides and are reached directly by wave splash only during the highest tides or during storm conditions. Because of their typically small volume, these pools lack the buffered environment of most other marine habitats and experience rapid changes in temperature and salinity as a result of daily variations in air temperature, wind, precipitation, and incident solar radiation. In the light of these harsh physical conditions, the depauperate nature of the fauna of supralittoral pools is not surprising. Ricketts et al. (1968) have pointed out that most supralittoral animals are, in fact, semi-terrestrial; in addition to being able to survive long periods of time out of water, these species can move from pool to pool over dry land.

Not all supralittoral species, however, are semi-terrestrial. The harpacticoid copepod *Tigriopus californicus* is the most conspicuous exception on the central California coast. The tolerance of this species and its congeners to extremes in the physical environment has been widely noted (Fraser, 1935; Ranade, 1957; Vittor, 1971; Kasahara and Akiyama, 1976; and others) and life histories have been worked out (Fraser, 1936, and Koga, 1970). The natural history of members of this genus is briefly summarized below.

The genus *Tigriopus* is represented by seven species which are abundant in splash zone pools all over the world (Bradford, 1967). The life cycle consists of 6 naupliar and 6 copepodid stages, the sixth being the adult form (Egloff, 1966). Adult males clasp immature females with geniculate first antennae, but successful insemination cannot occur until after the females' terminal molt. Each female then lays a brood of eggs into a single egg sac which is retained until the eggs hatch as Stage I nauplii. The female then produces another egg sac fertilized by sperm stored from the original mate; as many as 12 successive broods may be produced in this manner with a mean of 300 eggs

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produced per female (Vittor, 1971). There is no evidence that later matings occur or that any one brood or successive broods from the same female result from multiple inseminations. Males, however, are capable of inseminating more than one female each (Egloff, 1966). Generation time (egg to egg) in the laboratory at 20 °C is about 24 days.

Tigriopus appears to feed primarily on detritus and/or the flora and fauna associated with detritus (for review see Kinne, 1977, p. 763). Cannibalism by adults on nauplii has been documented in high density cultures (Egloff, 1966). *Tigriopus* is highly restricted to those tide pools located in the high intertidal zone (Vittor, 1971); this restriction is obviously not physiological and appears to be the result of predation, particularly by cottid fishes (M. Dethier, University of Washington, unpublished). Though capable of swimming across thin films of water, *Tigriopus* cannot move more than a centimeter over dry rock. Colonization of newly filled splash pools may be aided by the movement of crabs; as many as 73 *T. californicus* have been found adhering to a single specimen of *Pachygrapsus crassipes,* an abundant supralittoral (and semi-terrestrial) crab (Egloff, 1966). However, most movement of copepods between pools occurs during and after periods of exceptional wave splash and rain, when many pools are temporarily connected by streams of water (J. Cooper, Hopkins Marine Station, unpublished). Because of the short generation time and the large number of eggs produced per female, populations within single pools can grow rapidly after colonization.

Tigriopus can withstand short exposures to temperatures in excess of 40 °C (Ranade, 1957), and can tolerate daily fluctuations of over 10 °C during the warm summer months. Salinity tolerances have been more completely documented: Egloff (1966) observed active copepods in a pool with 102 ‰ S, and demonstrated the recovery of inactive adults and ripe eggs upon return to seawater from a pool with 334 ‰ S. We have observed active *T. californicus* in pools with salinity as low as 6 ‰. This high tolerance to salinity extremes is matched by very few other invertebrates (Kinne, 1971).

Despite considerable interest in the genetics of *Tigriopus* in the 1950's (see Buzzati-Traverso, 1958), little genetic information concerning the genus is available. On the basis of between-family selection experiments on the sex ratio of broods, Ar-rushdi (1958) concluded that sex determination in *T. californicus* is polygenic. In later work on the cytogenetics of *Tigriopus* (Ar-rushdi, 1963), the 2n chromosomal complement for the genus was found to be 24, with no evidence found for the existence of sex chromosomes. Other workers (Egloff, 1966; Vacquier, 1962; Takeda, 1950; and others) have suggested that various environmental factors influence the sex ratio of *Tigriopus* broods.

Because it is easily cultured in the laboratory and has a short generation time, *Tigriopus* provides excellent material for a population and ecological genetic approach to the study of invertebrate adaptations to life in the intertidal zone. Our laboratory has undertaken a number of projects whose aim is the description and explication of major aspects of the population biology of *T. californicus.* It is hoped that such studies will also provide insight into the population and ecological genetics of other intertidal animals that are less easily studied. In this first paper we describe the techniques used in studying enzyme polymorphisms in *Tigriopus* and present data relevant to the understanding of the structure of populations along the central California coast. A general review on population genetics of marine animals has been published in Volume II of 'Marine Ecology' (Gooch, 1975).

MATERIALS AND METHODS

Field Collections

The work discussed here primarily concerns *Tigriopus californicus* populations sampled from nine

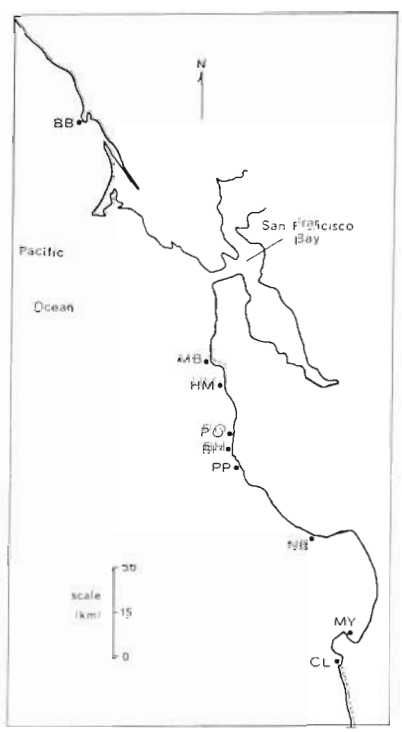

Fig. 1. Collection sites along the central California coast, USA. BB: Bodega Bay; MB: Moss Beach; HM: Half Moon Bay; PO: Pescadero; BH: Bean Hollow (Pebble Beach); PP: Pigeon Point; NB: Natural Bridges; MY: Monterey; CL: Carmel

sites along the central California coast between Bodega Bay to the north and Carmel to the south. The sites and the approximate distances between them are shown in Figure 1. The selection of field sites was made primarily on the basis of habitat availability for *T. californicus*. Habitat occurs in distinct patches where rock outcroppings allow the formation of supralittoral pools. Lack of habitat is responsible for most of the large gaps in our coverage of this section of coast; for example, Monterey Bay, which separates our Natural Bridges (NB) site from our Monterey (MY) site, has a nearly uninterrupted sandy shoreline without rocky outcroppings. Much of the area between Bodega Bay (BB) and Moss Beach (MB) consists of sandy beach; rocky habitat between these sites is largely inaccessible due to the presence of high rocky cliffs. Combined with the entrance to San Francisco Bay, these geographic features account for the large gap between the BB and MB sites.

In addition to the sites shown in Figure 1, one site in southern California (approximately 30 km northwest of Malibu), and two sites in Oregon (Battle Rock in southern Oregon and Yachats in central Oregon) were sampled and a limited amount of data obtained for comparison with the central California populations.

Population samples were collected from spray pools with a fine mesh aquarium net and maintained in approximately 250 ml of seawater collected from the same pool. An effort was made to collect copepods from throughout a given pool. For short-term maintenance of population samples, the collection vessels, 300–700 ml plastic bottles, were kept at room temperature (19 °–23 °C) and fed with small amounts of Tetramin brand flake-type fish food. Mortality was low (less than 5 %) among the adults for several weeks, during which time most electrophoretic analysis of the populations was completed. Long-term mass culture of *Tigriopus* was not required for this study, but is easily accomplished (Huizinga, 1971; Rothbard, 1976).

Electrophoresis

Individual adult *Tigriopus californicus* were homogenized by hand in 0.8 cm (diam) × 0.7 cm (deep) 'wells' in a Delrin (brand) plastic block using a Delrin rod. Each copepod was ground in 20 µl of 15 % sucrose in 0.1 M Tris-borate-EDTA buffer (pH 8.9) with enough bromphenyl blue (tracking dye) to lightly color the sample. The entire homogenate was immediately applied to a vertical polyacrylamide slab gel; gel concentration was 7 % for esterase (EST) and leucine aminopeptidase (LAP) gels and 5 % for phosphoglucose isomerase (PGI) gels. The acrylamide monomer to cross-linker ratio was 95 : 5 for all gels. Gels were run at 120 V until the tracking dye migrated about 9 cm for EST and LAP or for 20 minutes after the dye ran out the end of the 10 cm gel for PGI. Stain recipes followed Shaw and Prasad (1970) for LAP and PGI and Ayala et al. (1972) for EST.

Survey of Population Phenotypes

Copepods from each population studied were randomly sampled from the field collections. Only adult males and females were assayed; egg sacs were removed from all females. Inter-population comparisons were facilitated by including specimens from two or more populations on every gel.

Genetic Analysis of Electrophoretic Phenotypes

In order to establish the mode of inheritance for the electrophoretic markers used in this study, the phenotypes of offspring from parents with known phenotypes were determined. When preliminary screening showed that alternate phenotypes were fairly common within a population, the following procedure ('clasped-pair technique') was used: (1) Individual clasped-pairs of copepods were isolated in small compartments (petri dishes or plastic boxes) containing 40–60 ml of filtered seawater innoculated with a few drops of a unicellular green algal suspension prepared by filtering seawater from a tide pool containing dense algal growth; a few milligrams of finely-ground fish food were added to each compartment. (2) Cultures were maintained at room temperature (19 °–23 °C) under 24-h fluorescent illumination. (3) Cultures were checked on alternate days until the female of a pair produced an egg sac; at this time, the male was removed for electrophoretic analysis. (4) After the eggs hatched, the female was removed for analysis. If few nauplii were observed after the hatching of the first egg sac, the female was allowed to produce additional egg sacs before being sacrificed. (5) As the offspring matured, they were removed for analysis.

When initial screening showed the presence of a relatively rare phenotype, the above approach proved to be too laborious because few of the matings obtained by the clasped-pair technique involved the rare phenotype. To overcome this problem, we used the following procedure ('egg-sac removal'): (1) Females carrying ripe egg sacs, characterized by the red-orange color of the eggs, were pipetted onto filter paper. (2) Egg sacs were dissected from the females and placed individually into 1 ml of filtered seawater (a plastic 'mini' ice cube tray served to hold 90 isolated

egg sacs). (3) The females were then analyzed electrophoretically and those with the rare phenotypes identified. (4) Eggs previously dissected from females with rare phenotypes were transferred to petri dishes of filtered seawater inoculated with unicellular algae and allowed to mature. (5) Some of the mature adults (f_1 generation) were analyzed to test for segregation of the rare phenotype; since these were rare phenotypes in the field population, it was inferred that the f_1 progeny were the result of a heterozygote × homozygote (common type) mating, and 1 : 1 progeny ratios (homozygote [common type] : heterozygote) were expected. Other members of the brood were allowed to sib-mate, and the clasped-pair technique was used to analyze the progeny of these matings which now took place in a population (i.e., the single brood) enriched with the rare phenotype. Of particular interest were the heterozygote × heterozygote matings, from which 1 : 2 : 1 progeny ratios were expected, including 25 % of the rare-type homozygote, the phenotype that was most uncommon in nature.

Nomenclature

Electrophoretic phenotypes will be addressed in this paper by the enzyme system and upper case letter designations describing the relative anodal mobility of the bands observed (e.g. esterase F/S had a fast and a slow migrating band, esterase F had the fast band only, and esterase N had no esterase band in the region scored [= null]). When referring to the genetics of specific alleles, a three letter code for the enzyme system and a lower case superscript denoting allelic mobility is used (e.g. Est^f = esterase fast allele, Lap^s = leucine aminopeptidase slow allele, Pgi^m = middle-running phosphoglucose isomerase allele). For convenience, we refer to the observed 'electromorphs' as alleles. The possibility that electrophoretically cryptic variation (e.g. Bernstein et al., 1973; Singh et al., 1976) exists within mobility classes has not yet been investigated.

RESULTS

Esterase

Three distinct regions of esterase activity appeared on gels with α-naphthyl acetate as substrate; only the fastest migrating (most anodal) region, which stained most intensely, was studied. Four phenotypes, including a 'null' phenotype, were observed in the central California sites. The genetic basis of these phenotypes was established by using the clasped-pair technique on population samples from Moss Beach (Table 1). The four phenotypes are the result of a three allele polymorphism. The relative mobility of the alleles standardized to the most common form are Est^f = 1.00, Est^s = .97, with no band observed for Est^n. The 'F' phenotype is observed among individuals of either Est^f/Est^f or Est^f/Est^n genotypes, the 'S' phenotype is observed among Est^s/Est^s and Est^s/Est^n individuals, 'F/S' among Est^f/Est^s individuals only, and 'N' among Est^n/Est^n individuals only. Est^f/Est^s individuals had two esterase bands, suggesting a monomeric structure for the active *in vitro* enzyme.

When Est^n is relatively common, the frequencies of the esterase alleles in polymorphic populations can only be estimated; this situation is analogous to the ABO blood group system in humans where the '0' allele is recessive and common. Elandt-Johnson (1971) describes three techniques for the estimation of allele frequencies in these systems.

Because of its computational simplicity and superior statistical efficiency, we have chosen to present the

Table 1. Esterase pair crosses

No.	Mating Phenotypes male	×	female	Progeny Phenotypes F	F/S	S	N	Inferred Genotypes male	×	female
BB1	F/S		F	15	9	0	0	Est^f/Est^s		Est^f/Est^f
BB5	S		F	4	1	3	4	Est^s/Est^n		Est^f/Est^n
BB6	S		N	0	0	10	6	Est^s/Est^n		Est^n/Est^n
BB9	F		F/S	27	10	8	0	Est^f/Est^n		Est^f/Est^s
BB10	F		F	27	0	0	10	Est^f/Est^n		Est^f/Est^n
BB12	N		N	0	0	0	30	Est^n/Est^n		Est^n/Est^n
F3	F/S		F/S	14	21	13	0	Est^f/Est^s		Est^f/Est^s
A3	F/S		S	4	7	15	0	Est^f/Est^s		Est^s/Est^n
B10*	S		S	0	0	18	0	$Est^s/Est^{(s\ or\ n)}$		Est^s/Est^s
Pes*	F		F	25	0	0	0	$Est^f/Est^{(f\ or\ n)}$		Est^f/Est^f

* Genotypes involved in these matings cannot be completely inferred; one of the four genes (either from the male or the female) may have been Est^n.

gene counting estimates of the electromorph frequencies in Tables 2, 3, and 4. We have also calculated the adjusted Bernstein estimates (Elandt-Johnson, 1971) for the same data and found them to be essentially identical to the gene counting estimates.

The geographic and temporal distribution of esterase phenotypes and estimated allele frequencies is presented in Table 2. Only the three most northern sites show consistent polymorphism for esterase phenotypes. The polymorphism is most pronounced at Moss Beach, where the frequency of each allele always exceeds .08 and usually exceeds .20. Because of the recessive nature of the Est^n allele, the possibility that it occurs in heterozygous form in the populations where only the F phenotype was observed cannot be excluded; the single N individuals observed in the 1978 Pigeon Point and Natural Bridges samples indicate that Est^n may be widespread but at low frequency.

Table 2. Spatial and temporal distribution of esterase phenotypes and allele frequency estimates. (Gene counting method)

Site	Date		N	Phenotype frequencies				Est. frequencies		
				F	F/S	S	N	Est^f	Est^s	Est^n
Bodega Bay	Jan	78	152	0.960	0.026	0.013	0.000	0.911	0.020	0.068
	Feb	78	212	0.976	0.005	0.005	0.014	0.868	0.005	0.127
	June	78	67	0.925	0.030	0.045	0.000	0.831	0.040	0.129
Moss Beach										
Pool S	May	76	91	0.286	0.286	0.330	0.100	0.338	0.372	0.290
Pool F	May	76	546	0.377	0.150	0.370	0.103	0.322	0.316	0.362
Pool SB	May	76	54	0.407	0.204	0.352	0.037	0.394	0.349	0.257
Pool F	July	77	268	0.284	0.310	0.261	0.145	0.346	0.329	0.325
Pool N	Aug	77	90	0.511	0.344	0.078	0.067	0.583	0.226	0.187
Pool N	Nov	77	222	0.315	0.495	0.072	0.117	0.495	0.300	0.190
Pool N	Feb	78	117	0.308	0.598	0.017	0.077	0.572	0.313	0.084
Pool R	Feb	78	122	0.443	0.229	0.246	0.082	0.430	0.277	0.293
Pool F	May	78	119	0.260	0.277	0.344	0.118	0.312	0.375	0.312
Pool C	May	78	73	0.247	0.260	0.411	0.082	0.296	0.424	0.280
Pool N	July	78	83	0.313	0.289	0.301	0.096	0.362	0.353	0.285
Pool N	Dec	78	107	0.327	0.215	0.346	0.112	0.324	0.338	0.338
Half Moon	July	77	210	0.886	0.100	0.009	0.005	0.876	0.056	0.068
Bay	Aug	77	40	0.975	0.025	0.000	0.000	0.987	0.013	0.000
	June	78	142	0.922	0.000	0.000	0.078	0.722	0.000	0.278
Pescadero	Jan	76	75	1.0				1.0		
	Mar	76	56	1.0				1.0		
	July	77	50	1.0				1.0		
	Jan	78	95	1.0				1.0		
	Feb	78	120	1.0				1.0		
	May	78	141	0.993	0.007			0.996	0.004	
	June	78	91	1.0				1.0		
Bean	Mar	76	59	1.0				1.0		
Hollow	July	77	80	1.0				1.0		
	June	78	42	1.0				1.0		
Pigeon	Mar	76	12	1.0				1.0		
Point	July	77	79	1.0				1.0		
	Jan	78	236	1.0				1.0		
	June	78	71	0.986			0.014	0.881		0.119
Natural	July	77	60	1.0				1.0		
Bridges	Jan	78	161	0.994	0.006			0.997	0.003	
	Mar	78	146	0.993			0.007	0.917		0.083
	June	78	40	1.0				1.0		
Monterey	June	76	79	1.0				1.0		
	July	77	61	1.0				1.0		
	July	78	30	1.0				1.0		
Carmel	June	76	87	1.0				1.0		
	July	77	97	1.0				1.0		
	Mar	78	94	1.0				1.0		
	July	78	56	1.0				1.0		
Battle Rock (Oregon)	Aug	78	60	1.0				1.0		
Yachats (Oregon)	Aug	78	60	0.983			0.017	0.871		0.129

Because of the localized nature of the esterase polymorphism discovered in 1976, a more detailed study of the Moss Beach site was initiated. Figure 2 shows the relative locations of the pools referred to in Tables 2, 3 and 4. In order to establish whether or not there were significant differences in phenotype frequencies among pools at a given time, seven pools were sampled in December, 1978. Phenotypic frequencies were determined from electrophoretic analysis of approximately 100 adults from each pool (Table 3). A seven (pools) by four (phenotypes) test of independence using the G-test indicated that there were highly significant differences among pools ($G = 59.76$, and for the corresponding χ^2_{18}, $P < .005$). Despite the statistical significance of this test on the phenotypes, it should be noted that the allele frequencies among the pools are qualitatively similar; only in Pool R does an allele frequency exceed .410 or go below .245. The three pools located closest to each other (W, R, and SB) did not differ among themselves ($G = 8.44$, $df = 6$, $.5 > p > .1$). Nor, however, did the two pools (S and FN) located furthest apart ($G = 6.22$, $df = 3$, $.1 > p > .05$). Three of the pools (W, R, and S) differ significantly from Hardy-Weinberg expected phenotype frequencies.

To test for temporal changes in phenotype frequen-

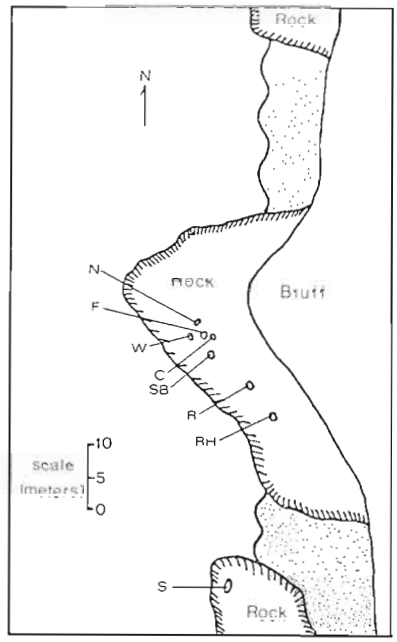

Fig. 2. Relative locations of pools at the Moss Beach site. Stippled regions: sandy beaches. Pool FN was located approximately 60 m beyond the northern boundary of the figure. Only pools for which data are presented are shown; other pools, some with *Tigriopus californicus* populations, were present within the study site

Table 3. Esterase phenotypes and estimated allele frequencies for seven Moss Beach pools sampled 4 December 1978

Pool	Sample Size	Phenotype frequencies F	F/S	S	N	Est. frequencies* Est^f	Est^s	Est^n	χ^2 fit to H-W equilib.
N	107	0.327	0.215	0.346	0.112	0.324	0.338	0.338	0.018; $p > 0.5$
W	99	0.252	0.364	0.293	0.091	0.361	0.393	0.245	5.60; $p < 0.025$
SB	108	0.268	0.250	0.315	0.167	0.298	0.331	0.370	3.28; $p > 0.05$
RH	100	0.290	0.230	0.320	0.160	0.302	0.323	0.375	1.34; $p > 0.1$
R	99	0.313	0.525	0.071	0.091	0.513	0.315	0.172	37.6 ; $p < 0.005$
S	100	0.270	0.430	0.170	0.130	0.410	0.333	0.257	22.8 ; $p < 0.005$
FN	105	0.352	0.286	0.257	0.105	0.389	0.316	0.294	1.60; $p > 0.1$

* Allele frequency estimated using the method of gene counting as described by Elandt-Johnson (1971).

Table 4. Esterase phenotypes and estimated allele frequencies for 1977–1978 samples of Moss Beach Pool N population

Date	Sample Size	Phenotype frequencies F	F/S	S	N	Est. frequencies** Est^f	Est^s	Est^n	χ^2 fit to H-W equilib
9 July 77	116	0.293	0.241	0.345	0.121	0.314	0.353	0.333	0.41; $p > 0.5$
20 Aug 77	90	0.511	0.344	0.078	0.067	0.581	0.225	0.194	7.17; $p < 0.01$
10 Nov 77	222	0.315	0.495	0.072	0.117	0.490	0.299	0.211	27.3 ; $p < 0.00$
13 Feb 78	117	0.308	0.599	0.017	0.077	0.558	0.312	0.130	63.0 ; $p < 0.00$
12 Apr 78	39	0.205	0.692	0.000	0.103	0.515	0.346	0.139	38.8 ; $p < 0.00$
21 June 78	47	0.404	0.340	0.064	0.191	0.454	0.210	0.337	12.8 ; $p < 0.00$
6 July 78*	73	0.370	0.192	0.219	0.219	0.331	0.228	0.440	1.55; $p > 0.1$
19 July 78*	83	0.313	0.289	0.301	0.096	0.362	0.353	0.285	0.90; $p > 0.1$
4 Dec 78	107	0.327	0.215	0.346	0.112	0.324	0.338	0.338	0.02; $p > 0.5$

* Pool known to have been completely dry on July 10.
** Allele frequency estimates using the method of gene counting as described by Elandt-Johnson (1971)

Table 5. Phosphoglucose isomerase pair crosses

	Mating phenotypes male × female		Progeny phenotypes F	F/M	M	Inferred genotypes male × female	
R7	F	M	0	10	0	Pgi^f/Pgi^f	Pgi^m/Pgi^m
Z4	F	F	7	0	0	Pgi^f/Pgi^f	Pgi^f/Pgi^f
BB1	F/M	F	4	6	0	Pgi^f/Pgi^m	Pgi^f/Pgi^f
BB8	M	F/M	0	15	18	Pgi^m/Pgi^m	Pgi^f/Pgi^m
BB10	F	F	17	0	0	Pgi^f/Pgi^f	Pgi^f/Pgi^f
BB4	M	M	0	0	7	Pgi^m/Pgi^m	Pgi^m/Pgi^m

cies, a series of nine samples were taken from the same pool (N) over a seventeen month period (Table 4). Differences in phenotype frequencies among the samples were highly significant. ($G = 181.6$, $df = 24$, $p < .005$). This pool was observed to be completely dry on July 14, 1978. The pool had refilled with water and had a population in excess of 3000 adult *Tigriopus californicus* when examined on July 19. A mildly significant difference was observed among the phenotype frequencies of the July 6 and July 19 samples ($G = 6{,}75$, $df = 3$, $.05 > p > .025$).

As shown in Table 2, the Est^f allele is predominant in the Oregon samples, with Est^n also present in the Yachats sample. The Malibu sample appeared to be fixed on a fourth allele with relative mobility between Est^f and Est^s, but attempts to establish the genetics of this phenotype by crossing to Moss Beach animals failed when the Malibu sample was lost from culture.

Phosphoglucose Isomerase

Four PGI phenotypes have been observed in the central California populations. Using the clasped-pair technique, the genetic basis of the three most common types has been studied (Table 5). These phenotypes are due to the segregation of two alleles Pgi^m and Pgi^f. The heterozygote Pgi^m/Pgi^f has a three-banded pattern suggestive of a dimeric enzyme structure. While analysis of the fourth phenotype, S/M, has not been completed, it is also a three-banded type with its fastest band co-migrating with the Pgi^m band, indicating that it is produced by a heterozygote Pgi^m/Pgi^s genotype,

Table 6. Temporal and spatial distribution of PGI phenotypes

Site	Date		N	Phenotype frequencies F	F/M	M	M/S	S	Allele frequencies Pgi^f	Pgi^m	Pgi^s
Yachats	July	78	27			1.0				1.0	
Bodega	May	78	40			1.0				1.0	
Moss Beach											
Pool FN	Apr	78	70			1.0				1.0	
Pool N	Apr	78	40			0.950	0.050			0.975	0.025
	June	78	40			1.0				1.0	
	Dec	78	40			1.0				1.0	
Pool F	May	78	45			1.0				1.0	
	Aug	78	45			0.890	0.110			0.944	0.056
Half Moon	June	78	65			1.0				1.0	
Bay	Aug	78	65			1.0				1.0	
Pescadero	Apr	78	50	0.320	0.400	0.280			0.520	0.480	
	June	78	84	0.143	0.464	0.381		0.012	0.375	0.613	0.012
	Aug	78	104	0.260	0.480	0.260			0.500	0.500	
Y-Site	Aug	78	100		0.070	0.830	0.100		0.035	0.915	0.050
Bean	June	78	25			0.960	0.040			0.980	0.020
Hollow	Aug	78	105		0.067	0.895	0.038		0.033	0.948	0.019
Pigeon	June	78	22			0.955	0.045			0.977	0.023
Point											
Natural	Mar	78	60			1.0				1.0	
Bridges	June	78	35			1.0				1.0	
Monterey	July	78	35			0.071	0.029			0.986	0.014
Carmel	July	78	75			0.853	0.147			0.927	0.073
Malibu	Mar	78	87			0.161	0.437	0.402		0.379	0.621

where Pgi^s is a less common third allele. The Pgi^s allele was common only in the southern California (Malibu) sample, where 40 % of the sample consisted of Pgi^s homozygotes. The relative mobilities of the PGI alleles are Pgi^m = 1.00, Pgi^f = 1.05, and Pgi^s = 0.91.

The PGI phenotypic distributions among populations studied are presented in Table 6. April and June, 1978, samples of the central California populations suggested that the Pgi^m allele was nearly fixed in all of these populations except Pescadero. The late August collections at Pescadero and neighboring sites (Pigeon Point through Moss Beach) confirmed the restricted nature of this polymorphism. The Pgi^f frequency at Pescadero was .5, while at the neighboring Bean Hollow (3 km to the south), the frequency of this allele was only .033. An additional site (Y-Site), approximately equidistant from Pescadero and Bean Hollow, was sampled; here the Pgi^f frequency was .035, indicating that the rapid change in allele frequency occurs somewhere over the 1.5 km distance that separates the Y-Site from the Pescadero site. We are currently monitoring the stability of this localized polymorphism.

Leucine-aminopeptidase

No strong polymorphism has been observed for LAP at any of the central California sites; i.e., the frequency of the second most common allele is never greater than .05. As many as five phenotypes have been observed in a single sample from Bodega Bay. Three of these have been determined to be the result of a two allele polymorphism via genetic analysis using the egg-sac removal technique. The progeny of a LAP F/S female were 8 : 14 (S phenotype : F/S). A sib-mating of two F/S individuals produced 6 : 11 : 4 (S : F/S : F). These data are consistent with the segregation of Lap^s and Lap^f alleles. The heterozygote has a two-banded phenotype, suggesting a monomeric form for the active *in vitro* enzyme. The mobility of Lap^f relative to Lap^s is 1.04.

The distribution of LAP phenotypes among the central California populations is presented in Table 7. Included are phenotype frequencies of forms that have not been genetically analyzed. The single southern California population sampled was fixed (N = 78) on an allele that migrated slightly faster than the Lap^s form in central California. Genetic analysis of this morph was not completed as the Malibu sample was lost from culture.

DISCUSSION

The complex life histories of many marine invertebrates makes formal genetic analysis of phenotypic

Table 7. Spatial distribution of LAP phenotypes and apparent frequency of the Lap^s allele

Site	Date		N	Phenotype frequencies						$Lap^{s\bullet\bullet}$
				S	F/S	F	S/VS	VS	Other*	
Bodega	Jan	78	90	0.995	0.033	0.011				0.972
	Feb	78	196	0.964	0.031				0.005	0.982
	June	78	92	0.902	0.054	0.011			0.032	0.946
Moss Beach										
Pool N	Dec	77	80	1.0						1.0
	Feb	78	130	0.084			0.008		0.008	0.992
	Dec	78	60	1.0						1.0
Pool F	Feb	78	20	1.0						1.0
	Apr	78	81	1.0						1.0
	July	78	100	1.0						1.0
Half Moon Bay	June	78	99	1.0						1.0
Pescadero	Feb	78	20	1.0						1.0
	Apr	78	89	1.0						1.0
Bean Hollow	June	78	30	1.0						1.0
Pigeon Point	June	78	60	1.0						1.0
Natural Bridges	Mar	78	166	0.958			0.030	0.012		0.973
Monterey	July	78	20	1.0						1.0
Carmel	Mar	78	65	1.0						1.0
	July	78	20	1.0						1.0

* All phenotypes in this category had one band that co-migrated with the Lap^s band.
** Calculation assumed that individuals in 'Other' category are heterozygous for the Lap^s allele.

variation in these species difficult. Hence, while the use of electrophoresis has permitted large-scale investigation of isozyme variation among intertidal invertebrates (for review see Gooch, 1975), few investigators have provided convincing evidence that the observed variation is in fact the result of segregating alleles. Numerous reports in the literature show that a variety of non-Mendelian processes can result in apparent isozyme variation (e.g. Gill, 1978a, b). This work indicates that all electrophoretic analysis not accompanied by formal genetic studies must be interpreted cautiously.

Previous studies concerning the genetics of marine copepods have been largely restricted to the analysis of quantitative characters such as size, development time, physiological tolerances (McLaren and Corkett, 1978; others) and, in the case of *Tigriopus californicus*, sex ratio (Ar-rushdi, 1958). These studies provide important insight into the relative roles of genetic variation and phenotypic plasticity in determining population characteristics. Questions concerning population genetic structure, however, are more easily addressed via studies of characters with simpler modes of inheritance. Among copepods, simple Mendelian color polymorphisms have been studied extensively among species of the genus *Tisbe* (reviewed by Gooch, 1975). Biochemical genetic techniques, however, have not been widely applied to copepods. Gooch (1977) found *Tisbe* individuals to be too small for electrophoretic analysis using his starch microgel system; bands were obtained from only two enzyme stains, malic dehydrogenase and phosphoglucose isomerase. Manwell et al. (1967), successfully applied electrophoretic techniques to differentiate between two *Calanus* species whose taxonomic status was not clear. While polymorphism among individuals within species was observed for esterases and malic dehydrogenase, the genetic basis of the observed variation was not studied.

The techniques and results discussed in this paper demonstrate the relative ease with which the formal genetic analysis of electrophoretic polymorphisms in *Tigriopus* can be completed. In addition to the three enzyme systems discussed here, we have initiated studies of a number of other systems which yield scorable banding patterns from homogenates of single adult copepods.

The results of our survey of esterase, phosphoglucose isomerase, and leucine aminopeptidase phenotypes among central California populations give some insight into the genetic structure of *Tigriopus* populations in this region. The abrupt change in PGI allele frequencies between the Y-Site and Pescadero indicates strong population differentiation over a 1.5 kilometer stretch of coastline. Similarly, the three allele esterase polymorphism, which is largely restricted to Moss Beach, demonstrates sharp differentiation between this population and that occupying the nearest neighboring habitat, located 15 km to the south. The low levels of variation observed for LAP were also restricted to local populations.

Our only extensive data dealing with genetic variation among pools within a habitat patch concerns the esterase polymorphism at Moss Beach. Significant differences in phenotypic frequencies have been documented both among several pools sampled simultaneously and within a single pool sampled repeatedly over time. While statistically significant, it should be noted that the differences among samples show no clear temporal or microgeographic patterns. In fact, with respect to estimated allele frequencies, most of the Moss Beach population samples are qualitatively similar. No significant differences were observed among the phenotype frequencies of three pools located within five meters of one another when sampled in December, 1978. This might have been expected since the observation has been made that *Tigriopus californicus* can migrate among pools located within a few meters of one another as described above. The fact that no significant difference could be demonstrated between the two Moss Beach pools located furthest apart (approximately 200 m) is somewhat unexpected; these pools are never connected by wave splash and it is improbable that any supralittoral crab movement occurs between them. This makes it difficult to interpret the overall heterogeneity.

Many of the pools inhabited by *Tigriopus californicus* completely evaporate and refill with water one or more times during the year. Complete evaporation results in death for all resident copepods. Neighboring pools within the habitat patch that have not completely dried out appear to be the source of adult copepods that recolonize newly refilled pools. The observed extinction and rapid recolonization of the Moss Beach Pool N population in July, 1978, indicates that within a habitat patch recolonization involves the movement of large numbers of adult copepods among pools after periods of high wave splash and rain. Alternatively, recolonization could result from the chance arrival of a single fertilized female carried by currents and wave action from another patch to the uninhabited pool. While this mechanism apparently operates when regional extinction occurs, it is probably rare, as *Tigriopus* has never (to our knowledge) been observed in the plankton (Vittor, 1971). Because large numbers of adults colonize recently refilled pools little evidence for a 'founder effect' is expected. This is in accord with our observations of esterase allele frequencies which were qualitatively similar for all Moss Beach pools

studied; of approximately 100 population samples representing 35 pools over the period of March, 1976, to December, 1978, none were fixed on one allele and all three alleles were observed in every pool from which 20 or more individuals were analyzed.

The statistical significance of the phenotypic frequency differences between the Pool N samples before drying and after recolonization, although weak, is difficult to interpret. We do not have data concerning the phenotypic distributions of neighboring pools during the recolonization event; the change in Pool N frequencies may therefore reflect heterogeneity among these pools. The change could also result from the fluctuations in pool salinity that accompanied this period of time. As the pool dried, salinity increased to S = 78 ‰ on July 6. If high salinity selectively favors one esterase phenotype over another, the observed change might be ascribed to the different salinity regimes experienced by neighboring pools. Salinity of 4 nearby pools never exceeded 48 ‰ in July, 1978 (personal observation); colonists arriving at the newly refilled Pool N would not then have similar phenotype frequencies as the earlier, high salinity, Pool N population. Such an explanation is completely speculative at this time. We plan to address the possible role of salinity as a selective agent on phenotype frequencies in the esterase system and other biochemical polymorphisms in future work.

A number of the population samples we studied had esterase phenotype frequencies that were not in accord with those expected on the basis of Hardy-Weinberg equilibrium. In all these samples, there were deficiencies of the 'S' phenotype and excesses of the 'F/S' type. This observation raises the possibility of heterozygote Est^f/Est^s superiority in overall fitness, although the role of population mixing cannot be excluded.

In Volume II of 'Marine Ecology', Gooch (1975) has reviewed the relationship between observed genetic differentiation of populations and dispersal ability among marine organisms. In general, species with long-lived pelagic larvae show little genetic differentiation over large geographic areas, while species with little or no larval dispersal tend to show considerably more differentiation. *Tigriopus californicus*, with strong differentiation observed over short geographic distance, appears to fit into this pattern. While the larval stages of *T californicus* could be subject to fairly broad dispersal during their development, the supralittoral ecology of the species as described above suggests that such dispersal does not commonly occur.

The existence of isolated pockets of polymorphism among *Tigriopus californicus* populations appears to be unique in the population genetic literature of marine organisms. Our data suggest that these pockets of polymorphism are stable in time in that sequential samples from each site are qualitatively similar. This pattern of variation contrasts sharply with the smooth clinal patterns more commonly observed among marine species. Furthermore, many of the observed clines are strongly correlated with temperature (e.g., Schopf and Gooch, 1971; Corbin, 1977) or salinity (Koehn et al., 1976), and it has been suggested that these environmental parameters may play a role in the maintenance of these polymorphisms. Similar correlations among allele frequencies and environmental parameters are certainly not obvious with respect to *T. californicus* allozyme polymorphisms. Such correlations would require sharp differences in environment over short stretches of coastline. The only environmental parameter that is likely to vary among habitat patches spaced as closely together as the Pescadero and Y-Site on the open coast is exposure of pools to wave splash. Exposure differences may affect the variability of many environmental parameters, especially pool salinity. However, *T. californicus* only occurs where wave splash is sufficient to occasionally fill supralittoral pools. Qualitative observations indicate that habitat patches do not differ significantly with respect to exposure. Clearly, further investigation is needed if we are to understand the unique patterns of genetic variation observed in *Tigriopus*.

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