

GENETIC VARIABILITY IN PALAEEMONETES PUGIO  
IN HABITATS OPEN AND CLOSED TO MIGRATION

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A Thesis

Presented to  
the Faculty of the Department of Biology  
University of Houston

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In Partial Fulfillment  
of the Requirements for the Degree  
Master of Science

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by

Belinda Fuller

December 1977

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Nine collections of the grass shrimp, Palaemonetes pugio, were made on Galveston Island to test the hypothesis that small populations show reduced genetic variability as a result of increased allele fixation due to random genetic drift. Four small ponds and five sites in or adjacent to the bay were sampled as representative of finite and infinite populations, respectively. Starch gel electrophoresis was used to analyze enzyme electromorphs encoded by seventeen loci. Four loci showed electromorph variation. Three measures of genetic variability were determined: percent polymorphism ( $\bar{P}$ ), the number of alleles per population for the polymorphic loci ( $n$ ), and the average heterozygosity per locus ( $\bar{H}$ ). For all three measures, the four closed populations had values lower than or equal to the smallest value found among the open populations. These results are believed to support the hypothesis that population size can be an important determinant of genetic variability resulting in reduced variability in small populations.

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## INTRODUCTION

Individuals within a sexually reproducing species display a wide range of heritable phenotypic variation. With the development and improvement of modern biochemical techniques, it is possible also to observe variation at the molecular level. Mechanisms to account for the presence and maintenance of genetic variability within species have been proposed, but there is a paucity of data from natural populations that is directly applicable to tests of the theories. Levels of variability in populations are a function of the proportion of polymorphic loci and the frequencies of different genotypes at these loci. The differences among populations within a species may be statistically described by gene frequency shifts at polymorphic loci.

Whether unique physiological characteristics are realized from the different alleles or combinations of alleles in natural populations has been a major point of dispute in population genetics. Amino acid sequencing has disclosed remarkable similarities among phyla in the rate of nucleotide substitution and in the amino acid sequence of several enzyme groups responsible for the same metabolic function in the organisms studied. This observation, along with an attempt to explain the high levels of genetic variability observed in natural populations, led to the

formulation of the neutral mutation hypothesis (Kimura, 1968). This hypothesis suggests that some of the mutant alleles that produce amino acid substitutions in protein do not alter the function of the enzyme and thus are selectively neutral, and that most of the variation observed is not adaptive. Amino acid substitutions away from the active site of an enzyme may not alter the three-dimensional configuration of the enzyme or its catalytic properties. Amino acid sequence differences have been identified in functional classes of enzymes such as cytochrome c, fibrinopeptides, and hemoglobins, which, as far as present techniques can ascertain, do not seem to alter the function of the molecule in vitro (King and Jukes, 1969). If some mutations are neutral, a large amount of variability could be retained (the amount depending on the mutation rate, size and age of the population) without affecting the adaptiveness of the organism or the population (Kimura, 1968).

One school of thought believes that most new mutations are deleterious and that evolution is a purifying and directional force, weeding out deleterious genes and increasing frequencies of advantageous ones (Muller, 1950; Lewontin, 1974). If the frequencies of alleles at most loci are primarily determined by directional selection, it would follow that all but the fittest alleles would be weeded out,

resulting in lower variability than would theoretically be obtained under other models.

Central to the neutralist-selectionist controversy is the question of the significance of genetic variability per se as an adaptive strategy of a population. Evolution can be thought of as the "process of successive transformation of the genetic structure of a population, not the individual" (Nei, 1975), or as a shifting equilibrium in the frequency distribution of alleles at many loci (Wright, 1931). Changes in variability could result in changes in the population's adaptive potential, but whether the direction of this change is positive or negative is another point of controversy. Wright (1931) proposes that the changes in genetic structure, even if nonadaptive, may allow the population to reach a higher "adaptive peak" after going through a less adaptive phase. Others maintain that the genetic structure of a population develops because it is a positive adaptation to the environment (Ford, 1964); Wright, 1970).

Several mechanisms for the maintenance of variability have been proposed, and each suggests an adaptive significance of genetic variability. Classic theory claims that most selection is directional, that there is a most fit genotype and that there is a tendency for the rest to be eliminated. Therefore, selection in this case would tend to reduce the genetic load in a population and concomitantly reduce variability (Muller, 1950). Consequently, most of the

observed variability predicted by this model is the result of selectively neutral alleles that produce no phenotypic differences and thus are not subject to selection (Lewontin, 1974).

However, if the allelic differences represent physiological differences, there must be some form of selection that maintains a high frequency of alternate alleles for such enzymes. A polymorphic locus that displays stable intermediate gene frequencies may be responding to a form of balancing selection, i.e. heterozygote superiority or stabilizing forces that select for a different allele under different conditions (diversifying selection) (Dobzhansky, 1970). Diversifying selection includes frequency-dependent selection and selection schemes in which selective values for alternate alleles change between ecological niches, generations, or sexes. Both types of selection, diversifying and balancing can maintain stable polymorphisms. The balancing selection theory then attributes the observed variability to deterministic pressures (i.e. selection and migration) of non-neutral alleles (Dobzhansky, 1955, 1970).

If the alleles are neutral, polymorphism cannot be maintained by deterministic factors and must result from a random process (Kimura, 1964; Wright, 1951; Nei, 1975). For

example, when a few individuals colonize a new area (founder effect) or there is a drastic reduction in population size (bottleneck), the genetic variability is expected to fall as a result of a sampling error. As the size of the bottleneck is reduced, the probability of loss of rare alleles increases, and as a result, the percent of polymorphic loci and average heterozygosity per locus are reduced. The loss in heterozygosity is not directly proportional to the loss of alleles and can occur when there is no loss of alleles (Nei, Maruyama, and Chakarabarty, 1975). The loss of variability in a bottleneck may be strictly a random process and may negate even strong deterministic pressures (Wright, 1931). Similarly, in a finite population, gene frequencies cannot be expected to remain constant in the absence of deterministic factors. The parental group for succeeding generations is always smaller than the total population size. Reproduction is analogous to sampling the population each generation and may result in changes in gene frequency (random genetic drift). Such sampling errors accumulated over time could produce gene frequencies far from the original values (Wright, 1931; Kimura, 1955; Nei, 1975).

If a few individuals do not reproduce in a large population, the sampling error may be insignificant, while if the same number do not reproduce in a small population, there is a larger error. In an infinitely large breeding

population ( $>10^6$ ), sampling errors of gametes are small, and gene frequencies tend to fluctuate around an equilibrium value that is determined by selection, mutation, and migration pressures (Wright, 1940; Crow and Kimura, 1970). However, in a small population (experiencing sampling errors at reproduction), alleles at intermediate frequencies will fluctuate randomly such that over a period of time the probability of their being in a frequency class near fixation ( $q=1.0$  or  $0$ ) increases (Wright, 1931). Thus, random genetic drift reduces genetic variability by decreasing the average number of alleles per locus and the percent of loci polymorphic in a population.

The population size considered is not the absolute number of individuals in the population, but effective population size ( $N_e$ ) (Nei, 1975). Effective size is reduced when reproduction is limited to only a portion of the population, there is differential fecundity or survival of offspring of different parents due to selective pressures or random causes (Wright, 1931), or differences in sex ratios. Also, even in large populations, individuals may tend to mate with nearby individuals, establishing neighborhoods with a high incidence of inbreeding. Partial isolation among neighborhoods occurs if the area occupied by the total population is large compared to the migration distance of an individual (isolation by distance) (Wright, 1941; Kimura and

Weiss, 1964) or if physical, ecological, or temporal barriers exist between colonies. This leads to a reduction in  $N_e$  (Wright, 1943) by inflating the level of inbreeding in the total population.

With sufficient isolation, local differentiation may occur in the absence of selection. Migration among subpopulations dilutes the effect of subdivision, retarding local differentiation, and producing one large panmictic population. Maruyama (1970) predicts that subdivision becomes biologically significant when the immigration rate per colony is less than one gamete/generation. It must be remembered that most migrants are from neighboring colonies with similar gene frequencies, so that the effect of migration will be reduced by a factor  $(1-r)$ , where  $r$  is the correlation between immigrants and the receiving groups (Wright, 1940). Consequently, for drift to be an important phenomenon, isolation between subpopulations must be sufficient to restrict migration (i.e.  $m < 1/2N_e$ , Crow and Kimura, 1970).

There have been several studies on experimental and natural populations uncovering reduced levels of variability compared to larger populations and implicating random genetic drift as the cause (Prout, 1954; Kerr and Wright, 1954; Neel and Ward, 1972; Lamotte, 1954). However, problems in determining the level of migration and ruling out selective pressures have rendered most of the studies inconclusive.

The purpose of this research is to examine several subpopulations of animals in which differences in  $N_e$  and isolation are maximized to test the predictions that variability decreases with decreasing  $N_e$ . It is necessary that the organism used is from a highly subdivided population with varying  $N_e$ 's in each subpopulation. The experimental organism should have a subdivided population structure inherent in which is a physical basis for estimating population size (e.g.  $N_e$  is directly proportional to habitat size). The best population arrangement would be similar to an island model (as defined by Wright, 1943) with restricted migration. In addition, the populations examined for evidence of drift should be small. It is then unnecessary to control for balancing selection and small changes in allele frequencies due to directional selection because the random drift hypothesis predicts that small size becomes more important than selection in determining gene frequencies. Also, the number of migrants finding (migration rate) or colonizing a habitat should be proportional to its size as a target area.

An easily accessible experimental arrangement exists on and near Galveston Island utilizing populations of the decapod Palaemonetes pugio that occur in the large, open bay network and small isolated ponds found on the island. This design is equivalent to an aquatic island model. Habitat size and separation distance between subpopulations are easily determined. Not only do I expect the pond (island) populations to



be smaller in absolute numbers (population size is assumed to be proportional to habitat size, because a finite habitat space can support a finite number of individuals), but I also expect that they are more vulnerable to periodic seasonal bottlenecks, further reducing  $N_e$ .

A grass shrimp, Palaemonetes pugio, is ubiquitous in Atlantic and Gulf Coast estuaries and exists in populations fitting the island design described above. This species has a range extending from northern Massachusetts to Texas (Holthuis, 1952). It occupies a wide variety of habitats, semi-closed to open, temporary to permanent, indicating that it is a good colonizer and can successfully exploit a wide variety of habitats. P. pugio tolerates salinities varying from 1-37 ppt, with 4-16 ppt being optimum (Wood, 1967). The optimal temperature range is 18-25°C (Wood, 1967). The life span of a single animal is approximately one year. There are usually two generations/year (one generation is 2-3 months, and the generations are overlapping) (Wood, 1967). Two spawning peaks and abundance peaks occur in July and October, although ovigerous females can be found from March to October (Wood, 1967; Knowlton and Williams, 1970). The larvae are planktonic (Knowlton and Williams, 1970) and are the primary dispersal stage. The adults prefer standing bodies of water with low current velocities (Antheunisse, Lammens, and Van Den Hoven, 1972). The population numbers

are found to drop in the winter, and the remaining shrimp in large water systems move to deeper waters to escape harsh conditions (Wood, 1967). This escape mechanism is not available in small ponds where the conditions may be more stringent.

Three species of Palaemonetes are reportedly found in the Galveston area: P. pugio, P. vulgaris, and P. intermedius (Wood, 1974). The three species overlap ecologically (Holthuis, 1952; Thorp and Hoss, 1975), referring to P. pugio and P. vulgaris); however, P. vulgaris was found to be less tolerant to low salinities than P. pugio (Knowlton and Williams, 1970; Thorp and Hoss, 1975; Bowler and Seidenberg, 1971). G. Penn (unpublished data) has found the occurrence of both species (P. pugio and P. vulgaris) in a single sample rare, even though both species inhabit the same area.

Genetic variability can be evaluated biochemically by electrophoresis. Electrophoresis is used to separate proteins so that differences in gene products and, therefore, differences in gene composition, can be visualized and qualitatively, as well as quantitatively, evaluated. Electrophoresis can theoretically detect approximately 1/3 of amino acid substitutions in proteins, in addition to separating proteins on gross size differences.

Data obtained from collections made in several habitats will be used to test the hypothesis that due to chance errors

when sampling gametes small isolated populations show less amount of genetic variability than large, open populations evidenced by fewer polymorphic loci, a smaller average number of alleles per locus, and lower average heterozygosity. The large populations should be genetically similar to each other, assuming gene frequencies are established by selection, migration, and mutation; but the small populations are expected to diverge from the large ones and from each other in a random, non-systematic manner. The degree of divergence should be proportional to habitat size (i.e. population size), distance from the large populations (Jaenike, 1973), and time since separation (Nei and Chakraborty, 1973). This is not a test of neutrality, but a study of the predictive value of the stochastic model.

#### MATERIALS AND METHODS

Palaemonetes pugio were collected from June through August, 1977, from ten locations (indicated in Figure 1). Four of these locations are small ponds, not connected to any other body of water. The remaining six are located in areas of the Galveston Bay system (localities, pond sizes, and salinity estimates are listed in Table 1).

Visually, the grass bed habitat appears continuous throughout the bays with no apparent obstruction to migration, so that an estimation of habitat size is unreasonable.

FIGURE 1

Collection Sites of Palaemonetes pugio on Galveston  
Island,

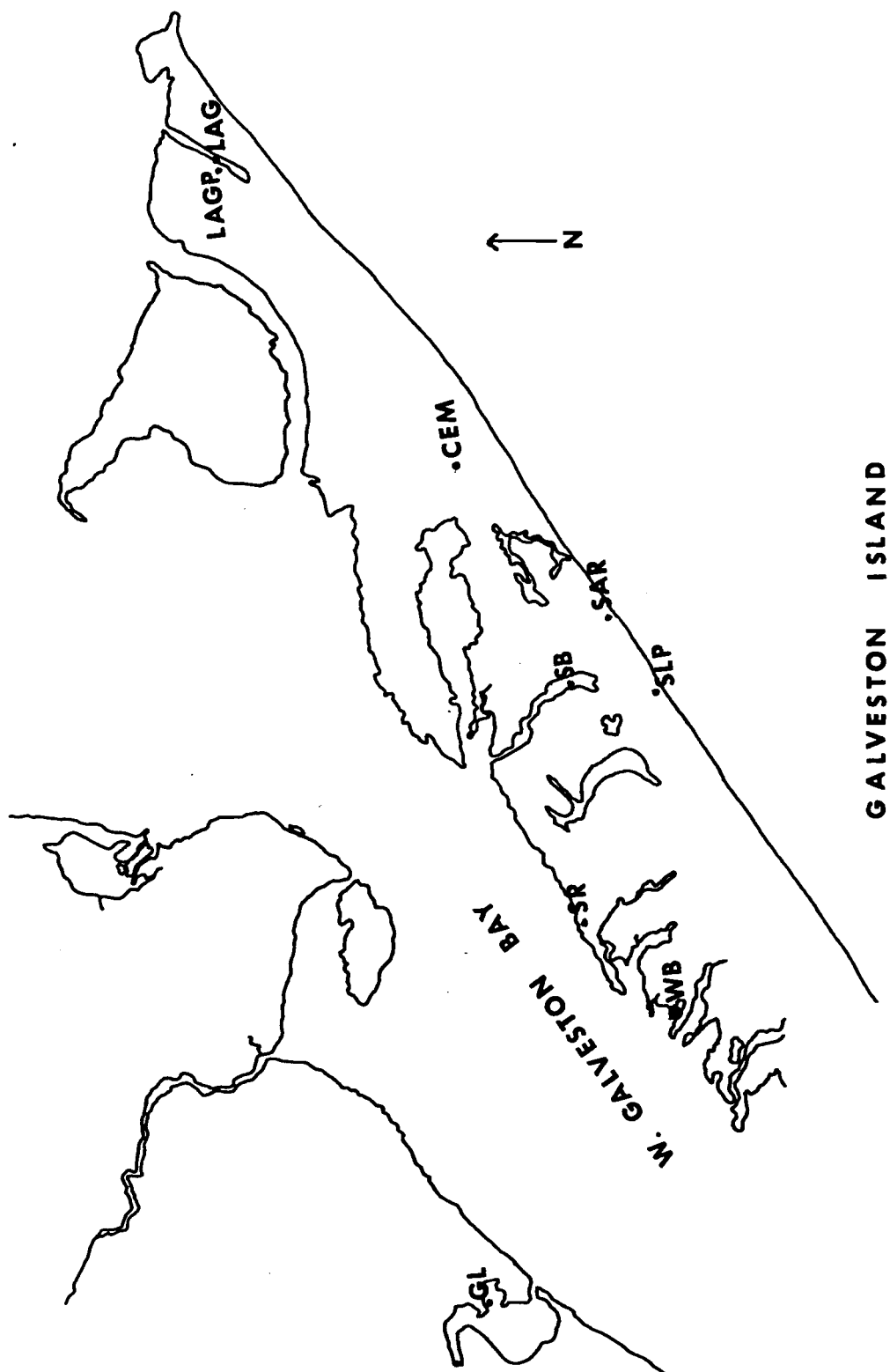


Table 1

Collection localities, approximate areas, and salinities.

<u>Locality*</u>	<u>Code</u>	<u>Area (m<sup>2</sup>)</u>	<u>Salinity (ppt)</u>
Lagoon pond (closed)	LagP	120	15-24
Lagoon (open)	Lag	-----	20-30
Cemetery pond (closed)	Cem	1,783	5
Sea Arama pond (closed)	SAr	24,232	4.5
Sydnor's Bayou (open)	SB	-----	22-35
San Luis Pass (closed)	SLP	42,525	7-15
Sportsman's Road (open)	SR	-----	23-52
West Bay (open)	WB	-----	24-34
Green's Lake (open)	GL	-----	-----

\*Localities are listed in order from east to west on Galveston Island.

Single samples were taken from each locality by pulling a small pushnet or a 15-foot small mesh (.25 inch diameter) seine for approximately five yards along the edge of the grass bed. Three pulls or more were made at each site (depending on species density) until an adequate sample was obtained. Several salinity measures were taken at each collection site with a refractometer. A rough estimate of the total area of each pond was made; the ponds were assumed rectangular in shape, and two sides were measured with a marked string. However, utilizable habitat area for P. pugio (area of grass beds) may not necessarily be directly proportional to total area of the pond.

One hundred shrimp were keyed to species on the basis of six morphological characters (Wood, 1974; Holthuis, 1952). These variables did not prove to be associated as described in the key or to fall into discrete classes and, therefore, were judged inadequate for separating P. pugio from two closely related sympatric species, P. vulgaris (Holthuis) and P. intermedius (Say). Strenth (personal communication) stated that better results would be obtained if secondary sexual characters were used. For this reason, 86 males were keyed on the basis of the number of setae on the tip of the appendix masculina: P. pugio - 5 setae; P. vulgaris and P. intermedius - 4 setae (Fleming, 1969), and on the rostral characteristics (characteristics 2, 3, and 4 above) with the

intention of correlating these with allozymic data to be obtained later.

All shrimp were frozen at  $-70^{\circ}\text{C}$ . Samples were thawed and refrozen to facilitate cell rupture and release of the intracellular enzymes. Before processing, eggs were removed from gravid females and all shrimp were washed in deionized water. Whole animals were ground for fifteen seconds in freshly made .2M Tris HCl - .0002M poly-vinyl pyrrolidone - .015M EDTA buffer (pH 8.0) using a motor driven teflon tissue homogenizer. The volume of homogenate added was approximately equal to total body volume of the sample. . Samples were centrifuged for thirty minutes at 19,500 rpm (46,300 g). The supernatant was pipetted into a culture tube and stored at  $-70^{\circ}\text{C}$  until use. This yielded enough fluid that the same samples could be used for multiple experiments.

Horizontal starch gel electrophoresis was employed to evaluate the genetic variability in the nine populations (Lewontin and Hubby, 1966). This method detects charge differences due to amino acid substitutions in protein molecules. Therefore, it can be used to differentiate the different charge forms of gene products of a single locus, known as allozymes (or electromorphs). A 13.25% solution of starch (Electrostarch, lot #307) and buffer were used. Seventeen samples plus an indicator dye (bromophenol blue)



were placed in each gel. In order to control for variation among gels and to standardize the scoring of polymorphic loci, samples from previous runs and from gels run the same day were included on each gel. Gels were stopped when the indicator dye reached the anodal margin. Gels were then sliced and each slice was used for a stain specific for a particular enzyme system. Each enzyme system (composed of all enzymes catalyzing the same reaction utilizing the same substrate, but sometimes coded for by more than one locus) was visualized on a zymogram which consisted of a banding pattern produced by enzyme activity at varying distances from the origin.

For zymograms representing gene products of multiple loci, the loci were numbered in ascending order corresponding to increasing migration distance from the origin. In scoring polymorphic loci (those containing isoalleles with different mobilities), the most common allele is designated M. Alleles migrating faster (more anodally) are designated F, with a numerical subscript identifying the faster migrating alleles by lower numbers. Alleles migrating slower than the common allele (more cathodally), are designated S, with the same rule for subscripts. Alleles possessing nearly identical electrophoretic mobility, such that multiple bands are not consistently distinguishable in the heterozygotes, were lumped into one electrophoretic mobility category

for most analysis. However, since the homozygotes were discernable, the alleles detected in homozygotes were counted for comparison of the average number of alleles possessed by a population.

Four gel and tray buffer systems were used in staining fifteen enzyme systems. All Buffer systems and stains were adapted from Selander et al. (1971), except mannose-6-phosphate isomerase and glycerate-2-dehydrogenase (Siciliano and Shaw, 1976), and hexokinase (adapted from Ayala et al., 1972). A list of recipes and stains is given below:

1. Tris citrate, pH 6.7 (gel); Tris citrate, pH 6.3 (tray). 150 v. Enzymes stained for: malate dehydrogenase (MDH, E.C. 1.1.1.37), lactate dehydrogenase (LDH, E.C. 1.1.2.3), isocitrate dehydrogenase (IDH, E.C. 1.1.1.42), glycerate-2-dehydrogenase (G-2-DH, E.C. 1.1.1.29), glutamic oxalacetic transaminase (GOT, E.C. 2.6.1.1).
2. Phosphate, pH 6.7 (gel and tray). 130 v. Enzyme systems: phosphoglucose isomerase (PGI, E.C. 5.3.1.9), phosphoglucomutase (PGM, E.C. 2.7.5.1).
3. Discontinuous Tris citrate (Poulik); Tris citrate, pH 8.7 (gel), pH 8.2 (tray). 250 v. Enzyme systems: Hexokinase (Hex, E.C. 2.7.1.1), alkaline phosphatase (Alk Ph, E.C. 3.1.3.1), soluble protein (AB), mannose-6-phosphate isomerase (MPI, E.C. 5.3.1.8).

4. Lithium hydroxide: LiOH a+B, pH 8.3 (gel); LiOH A, pH 8.2 (tray) 300 v. Enzyme systems: leucine amino peptidase (LAP, E.C. 3.4.1.1),  $\alpha$  naphthyl proprionate + Fast Blue RR salt ( $\alpha$ NP+FBRR, E.C. 3.1.1.1), naphthol AS-D acetate + FBRR (NADA, E.C. 3.1.1.1). (The same esterase system is scored from the last two stains to insure accuracy.)

(Loci are represented by small case letters and are underlined.)

If the observed electromorphs are gene products of alleles on chromosomes, the observed electrophoretic phenotypes at polymorphic loci should agree with Hardy-Weinberg expectations. Also the electromorphs of progeny from crosses should follow Mendelian segregation ratios. Two gravid females were placed in outdoor aquaria until the eggs hatched. The mothers were then frozen at  $-70^{\circ}\text{C}$ . The larvae, fed a diet of Artemia (Hubschman and Broad, 1974), required approximately two months to reach maturity (July - September). They were then collected and frozen. The mothers and broods were later used to test Mendalian inheritance of the polymorphic systems. If these expectations are met, then allozymes may be used for the estimation of measures of genetic variability (i.e. percent polymorphism, number of alleles, and average heterozygosity).

## RESULTS

Based on previously established electrophoretic criteria, seventeen zones of activity which were well resolved are assumed to represent the same number of loci. Four showed multiple allozymes and thus were polymorphic (Pgi, Pgm, Mpi, Nada), and thirteen were monomorphic. The observed genotypic distributions at the polymorphic loci in each population were tested for agreement with Hardy-Weinberg equilibrium. Rare genotypic classes were combined to yield an expected value suitable for a chi-square analysis.

The observed classes of Pgi, Pgm and Nada from two progeny groups agree with Mendelian expectations and offer no evidence that the electromorphs do not result from autosomal genes.

23.5% of the loci were polymorphic for all populations combined. Gene frequencies for the polymorphic loci are given in Table 2. In the LagP population (the smallest population), two of the polymorphic loci (Nada and Pgi) are fixed for the common allozyme, reducing percent polymorphism to 11.8%. The Nada locus is also fixed in the two other small populations (Cem and SAr) (% polymorphism = 17.6%). Therefore, the small populations closed to frequent migration show a lower percent of polymorphic loci. For the remainder of this paper, the pond populations will be referred to as

Table 2  
Gene frequencies in P. pugio

<u>Locus</u>	<u>Locality</u>								
	<u>LagP</u>	<u>Lag</u>	<u>CM</u>	<u>SR</u>	<u>SAr</u>	<u>SLP</u>	<u>GL</u>	<u>SB</u>	<u>WB</u>
<u>Pgi</u>	*(34)	(36)	(73)	(46)	(36)	(40)	(33)	(55)	(36)
F <sub>1</sub>		.01	.01				.03	.01	.01
F <sub>2</sub>		.01		.01	.03	.03	.015	.03	.10
M	1.00	.98	.99	.96	.97	.97	.88	.95	.88
S <sub>1</sub>				.02			.06		.01
S <sub>2</sub>				.01			.015	.01	
<u>Pgm</u>	(33)	(41)	(59)	(46)	(43)	(40)	(31)	(47)	(36)
F <sub>1</sub>				.022				.01	.01
F <sub>2</sub>	.88	.06	.29	.022	.30	.13	.06	.16	.03
M	.23	.915	.71	.89	.70	.86	.92	.78	.92
S <sub>1</sub>		.025		.033			.02	.04	.04
S <sub>2</sub>				.033		.01		.01	
<u>Mpi</u>	(31)	(48)	(41)	(41)	(30)	(40)	(33)	(39)	(35)
F <sub>0</sub>				.01				.01	
F <sub>1</sub>		.05		.12		.07	.07	.05	
F <sub>2</sub>		.14	.01	.11		.09	.11	.09	.10
F <sub>3</sub>	.10	.09	.07	.10	.05	.05	.17	.10	.16
M	.88	.72	.78	.65	.87	.79	.61	.70	.73
S <sub>1</sub>	.02		.10	.01	.07		.04	.01	.01
S <sub>2</sub>			.04		.02			.04	

\* Number in parenthesis indicates sample size.

(Continued next page)

Table 2 (Continued)

Gene frequencies in P. pugioLocus

	<u>LagP</u>	<u>Lag</u>	<u>CM</u>	<u>SR</u>	<u>SA</u>	<u>SLP</u>	<u>GL</u>	<u>SB</u>	<u>WB</u>
<u>Nada</u>	(33)	(34)	(53)	(34)	(37)	(30)	(31)	(27)	(35)
M	1.0	.54	1.0	.46	1.0	.70	.55	.63	.53
S		.46		.54		.30	.45	.37	.47

---

"closed" (to migration) and the bay populations as "open" (to migration).

The contingency test for heterogeneity ( $G_H$ ) was performed with observed genotype numbers for each polymorphic locus to determine if the populations were homogeneous in their genotypic distributions at these loci. It was decided that a  $\chi^2$  value with a probability less than .05 would indicate heterogeneity among the group of populations for that character (Sokal and Rohlf, 1969). Although it weakens the test, it was necessary to lump all rare genotypic classes (frequency < .05) into one category for the analysis. The results of this analysis are given in Table 3. The  $G_H$  values of the nine populations for all four polymorphic loci were significant ( $P < .005$ ). In order to ascertain whether the heterogeneity was attributable to the open populations or the closed populations, the contingency test was applied next to each group separately. At the Pgm locus, the closed populations were heterogeneous ( $P < .005$ ), primarily because of a rise in the frequency of the  $F_2$  allele in the LagP pond population. The open populations were not significantly heterogeneous for this locus. For Mpi, the closed populations fall into two groups based on frequency of rare allozymes and were heterogeneous. The open populations were again homogeneous. However, the  $G_H$  test is particularly insensitive for this locus because of the large number of rare

allozymes which had to be combined into one class. The frequency of the Pgi common allozyme is too high (frequency of  $M > .9$ ) to test for heterogeneity in the closed populations. However, the open populations are heterogeneous ( $P < .05$ ), thus accounting for some of the heterogeneity observed among all collections. For Nada, three of the four closed populations are monomorphic for the M allozyme, thus no contingency test was performed on these populations. The open populations are homogeneous as a group, and clearly show gene frequencies divergent from those in the closed populations. This could account for the large  $G_H$  value for the total population. The  $G_H$  data indicates that, in general, the open populations have similar genotypic frequencies, while the closed are a more heterogeneous group (where variation exists). Most of the heterogeneity observed appears to arise from the differences between open and closed populations.

Before any evaluation of genetic drift can be made, population subdivision must be substantiated. The  $G_H$  statistic shows that the nine populations are consistently heterogeneous with regard to genotypic distribution, indicating that the population is subdivided and differentiated. The degree of subdivision can be estimated by measuring the gene differentiation relative to the total population. Nei (1973) derived the coefficient of gene differentiation,  $G_{ST}$ ,



Table 3

$G_H$  values from the contingency test for heterogeneity

(Values are compared with a  $\chi^2$  distribution)

<u>Locus</u>	<u>Total</u>	<u>Closed Populations</u>	<u>Open Populations</u>
Nada	196.268	----	5.65
	d.f.=16 P<.005		d.f.=8 .9<P<.5
Pgi	25.748	----	11.588
	d.f.=8 P<.005		d.f.=4 P<.05
Pgm	126.232	63.636	15.44
	d.f.=16 P<.005	d.f.=6 P<.005	d.f.=8 .1<P<.05
Mpi	52.248	13.108	9.962
	d.f.=16 P<.005	d.f.=6 P<.005	d.f.=8 .5<P<.1

as generalization of Wright's  $F_{ST}$  for evaluating population subdivision.  $F_{ST}$  is theoretically the correlation between two gametes drawn at random from each subpopulation relative to the correlation between two gametes drawn at random from the total population (Nei, 1973). In practice  $F_{ST}$  is computed as a standardized variance of gene frequency.  $G_{ST}$  is equal to the weighted average of  $F_{ST}$  for alleles. It is actually the ratio of the average gene diversity between subpopulations to the average gene diversity in the total population ( $D_{ST}/H_T$ ) (Nei, 1973). A  $G_{ST}$  of .10 - .15 indicates subdivision comparable to the level of  $G_{ST}$ 's for natural populations that are known to be subdivided (Fuerst, personal communication). The  $G_{ST}$  obtained for the P. pugio populations, .157, supports the hypothesis that these populations are subdivided.

One of the most sensitive measures of genetic variability (that may be most directly related to bottlenecking) is the number of alleles at the polymorphic loci in a population. These values are compared in Table 4. Fixation of the M allele at Nada in three closed populations and of the Pgi M allele in the LagP population contribute to the reduction in the total number of alleles for the polymorphic loci in the closed populations. The greatest number of alleles in any single closed population is fifteen, which is equal to the smallest number of alleles observed in any open population.

Table 4  
Number of Alleles at Polymorphic Loci

<u>Closed Populations</u>	<u># of Alleles</u>	<u>Open Populations</u>	<u># of Alleles</u>
LagP	10	Lag	15
Cem	13	SB	20
SAr	12	SR	20
SLP	15	WB	16
	—	GL	<u>18</u>
	50		89
Average # of Alleles/ Closed Population = 12.5		Average # of Alleles/ Open Population = 17.8	

The mean number of alleles per population is lower for the closed populations than for the open populations. This suggests that this measure of variability is also correlated with population size.

Another estimate of genetic variability that is commonly used to compare populations and species is average heterozygosity locus, ( $\bar{H}$ ). This is computed by dividing the total number of expected heterozygous genotypes ( $H$ ) by the total number of expected homozygous genotypes ( $J$ ) times the number of loci ( $l$ ), ( $\Sigma H / [(\Sigma J)(l)]$ ). For the nine populations of P. pugio,  $\bar{H}=7.2\%$ . For comparisons among populations and loci, an intralocus  $H$  was calculated for each population (see Table 5). The intra-locus heterozygosity is a function of allele frequencies. In a population that is in Hardy-Weinberg equilibrium, as allele frequencies move closer to 1.0 or 0, the intralocus heterozygosity concomitantly decreases. Therefore, for Pgi (with the exception LagP), Nada, and Mpi, all four closed populations have lower heterozygosities. For Pgm, the frequencies are more intermediate in all closed populations. The frequency of the  $F_2$  allozyme of Pgm ranges from .13 to .88, while the range of  $F_2$  in open populations is .02-.16. Thus, the heterozygosities are higher in the closed populations. An average heterozygosity/population was used to compare variability among populations. These are listed according to increasing

Table 5

## Intra-locus Heterozygosities

<u>Locus</u>	<u>Locality</u>								
	<u>LagP</u>	<u>Cem</u>	<u>SAR</u>	<u>SLP</u>	<u>Lag</u>	<u>SB</u>	<u>SR</u>	<u>WB</u>	<u>GL</u>
<u>Pgi</u>	.0000	.0198	.0582	.0582	.0394	.0964	.0778	.2154	.2207
<u>Nada</u>	.0000	.0000	.0000	.4200	.4968	.4662	.4968	.4982	.4950
<u>Pgm</u>	.3542	.4118	.4200	.2434	.1585	.3642	.2048	.1510	.1492
<u>Mpi</u>	.2152	.3750	.2353	.3604	.4514	.4876	.5408	.4314	.5804

$\bar{H}$  in Table 6. The four closed populations show lower  $\bar{H}$  than the five open populations. The smallest population, LagP, has the lowest  $\bar{H}$ , while the largest closed population, SLP, has a higher  $\bar{H}$  than the other closed populations. Therefore, the  $\bar{H}$  of a population appears to be related to population size, or more correctly, habitat size.

Genetic subdivision among the subpopulations can be described using Nei's genetic distance measure ( $d$ ) (Nei, 1975). The genetic distance is a log transformation of the normalized identity of genes between two populations. Pair wise comparisons between all populations of standard genetic distance are given in Table 7. Genetic distance is a measure of gene difference between two populations which theoretically estimates the accumulated number of gene substitutions per locus (Nei, 1972). The distances for P. pugio are lowest in like comparisons, i.e. closed populations show a smaller distance value with other closed populations than with any open population, and the same is true for comparisons of open populations with open populations. Overall, the distances between open with other open populations are lower than the distances between closed and closed populations ( $\bar{D}_{cl \text{ vs } cl} = .012$ ;  $\bar{D}_{op \text{ vs } op} = .001$ ;  $\bar{D}_{cl \text{ vs } op} = .021$ ). The greatest distances are seen between open populations compared with closed populations. The SLP population, which comes from the largest closed habitat, shows smaller distances

Table 6  
Average Heterozygosity/Population

<u>Population</u>	<u><math>\bar{H}</math></u>	<u>Variance of Heterozygosity (total)</u>
LagP	.03349	.00954
SAr	.04197	.01283
Cem	.04745	.01702
SLP	.06365	.01875
Lag	.06742	.02500
WB	.07624	.02529
SR	.07766	.03030
SB	.08320	.02996
GL	.08502	.03312

Table 7

Genetic Distances between Populations  
of P. pugio (Unbiased Estimate)

	LagP	Cem	SAr	SLP	Lag	SB	SR	WB
Cem	.0145							
SAr	.0135	.0001						
SLP	.0313	.0075	.0076					
Lag	.0452	.0170	.0177	.0017				
SB	.0309	.0096	.0103	.0004	.0010			
SR	.0525	.0231	.0242	.0044	.0004	.0026		
WB	.0480	.0186	.0191	.0028	.0004	.0018	.0011	
GL	.0470	.0176	.0190	.0030	.0005	.0015	.0006	.0004



from the open populations than the other closed populations, but the genetic distance between SLP and each closed population is less than the D's between that closed population and all open populations. Genetic distance estimates place SLP intermediate in its genetic profile between open and closed populations.

The above results are reiterated in an examination of the correlation of homozygosities. These pair wise comparisons are given in Table 8. The highest correlation is seen in the comparisons of the open populations to the other open populations. The average pair wise comparisons between the closed populations (.794) were slightly higher than the comparisons between the open populations (.769), but both were much higher than the correlation between the two groups (.645). The SLP population is more highly correlated with the open populations than with the closed populations. In fact, one open population, SB, has a higher correlation with all the closed populations than SLP. This indicates that the two population groups show more similar homozygosities within the group than between the two groups. This is true for all the populations except SLP, which shows a higher correlation with all the open populations than with any of the closed populations.

Table 8  
Correlations of Homozygosities

	LagP	Cem	SAr	SLP	Lag	SB	SR	WB	GL
LagP	1.000								
Cem	.979	1.000							
SAr	.992	.967	1.000						
SLP	.596	.648	.584	1.000					
Lag	.465	.548	.443	.979	1.000				
SB	.690	.741	.682	.991	.954	1.000			
SR	.532	.622	.515	.980	.994	.970	1.000		
WB	.414	.502	.427	.951	.963	.932	.966	1.000	
GL	.455	.566	.459	.938	.962	.931	.977	.989	1.000

## DISCUSSION

Current mathematical theory and an increasing amount of experimental data suggest that effective population size ( $N_e$ ) may be the most important factor affecting genic variability in sexual populations (Nei, 1975; Crow and Kimura, 1970). The interaction of this term with other factors influencing gene frequency is quite complex and increases the difficulty of constructing models of population genetics. The influence of population size on genic variability is dependent on the magnitude of other pressures relative to  $N_e$ .

If the selection coefficients ( $s$ ), mutation rate ( $u$ ), and migration rate ( $m$ ), are small and  $N_e$  is small, nearly all loci are expected to be fixed, and an individual will be homozygous at nearly all loci. Alleles at a single locus are considered to be under vigorous selection when  $s \gg u \gg 1/4N_e$  (Wright, 1931; Crow and Kimura, 1970). In the case of strong directional selection at a single locus, the frequency of the favorable allele will approach fixation under these conditions in a large population. If selection is small in a large population ( $s$  not much greater than  $u$ ;  $u$  not much greater than  $1/4N_e$ ), the frequency of the favorable allele will be maintained with slight fluctuation around a mean value determined by the magnitude of the selection coefficient

and mutation rate. If gene frequency change is due to sampling error, the direction of change is random and may be nonadaptive (Wright, 1931; Kimura, 1964). In general, there is a marked tendency toward chance fixation of one allele if  $4N_e s$ ,  $4N_e u$ , and  $2N_e m$  are less than one, while fluctuations are negligible if these quantities are large (Wright, 1940; Crow and Kimura, 1970). The sampling of the small closed populations of P. pugio was designed to minimize the products of population size with  $s$ ,  $u$ , and  $m$  so that these last three factors would be negligible in determining gene frequencies. The open bay populations are assumed infinitely large, and levels of variability should be maintained by any one or all three of the above factors.

The overall probability distribution of gene frequency classes is time and size dependent. In a single locus model with two alleles in equal frequency in a finite, randomly mating population, all frequency classes between 0 and 1.0 at that locus are equally probable after  $2N$  ( $N$  = the number of breeding individuals) generations. These classes continue to decrease in probability at the rate  $1/2N$ , while the 0 and 1.0 classes continue to increase in probability. Concomitantly, the average heterozygosity per individual decreases at a rate of  $1/2N$  generation (Wright, 1931; Kimura, 1955). This rate of decrease is independent of number of loci and only dependent on

population size (Kimura, 1955).

In the case of recurrent mutations, this decrease in heterozygosity cannot proceed indefinitely as a point will be reached where the chance elimination of alleles will be balanced by the occurrence of new mutations (Wright, 1931; Kimura, 1968).

Following the isolation of a small population, there is an observed decrease in heterozygosity without appreciable fixation or loss of alleles. Mutation may replenish alleles, but in a population that remains small, this has little effect on raising average heterozygosity. Even after a population reaches a large size, the new mutations, whether adaptive or neutral, are in such low frequency that they do not initially contribute much to this average and are subject to strong sampling error (Nei, Maruyama, and Chakraborty, 1975).

Bottlenecking probably occurred during colonization of the closed ponds by P. pugio. Due to restricted migration and area limitations, the effective population size has most likely remained small, preventing the reestablishment of levels of variability comparable to the level maintained in the source population.

The effects of small population size and the outcomes mentioned above are further compounded and increased by an increase in matings between related individuals, increasing

the level of inbreeding. Inbreeding enhances genetic drift by reducing the effective population size. The level is dependent on the population size and the degree of isolation (i.e. migration rate) between colonies of a subdivided population (Crow and Kimura, 1970). In a sufficiently isolated subpopulation ( $m \leq 1$  gamete/colony, Maruyama, 1970), inbreeding may be pronounced and lead to an increase in homozygosity because of the genetic similarity of mating individuals. Even though each subpopulation may be mating randomly, if  $m$  is small the total population as a whole cannot be considered panmictic and local differentiation may occur as a result of inbreeding and random fluctuations in gene frequencies (Wright, 1940; Kimura and Weiss, 1964). If the population was initiated by a small number of founders, as suspected in P. pugio isolates, individuals in later generations are probably related to some degree (assuming no migration), and inbreeding is high.

The only reasonable way to determine whether natural populations are subdivided in the absence of migration data is to look for indications of genetic differentiation. The  $G_H$  and  $\bar{G}_{ST}$  statistics are used to detect genetic differences among populations. The values of both of these statistics for P. pugio suggest population subdivision. The  $G_H$  values for the four polymorphic loci in nine populations are all significant. The open populations have homogeneous

frequencies at three polymorphic loci, and are heterogeneous at the fourth, Pgi ( $p < .05$ ). The closed populations have heterogeneous frequencies at the two polymorphic loci with high enough frequencies of rare alleles to be tested (Mpi, Pgm). This shows that, in general, the open populations are more homogeneous than the closed populations. These results suggest that there is very little migration between the open and closed populations, and among the closed populations, but that migration is fairly common among the open populations or some pressure is maintaining gene frequencies at a similar value.

The  $G_{ST}$  value, which measures differences in gene frequencies, also indicates differentiation among the subpopulations. Paul Fuerst (unpublished data) supplied some  $\bar{G}_{ST}$ 's (from compiled data) for comparison with the  $\bar{G}_{ST}$  obtained for P. pugio. Interspecific studies showed  $\bar{G}_{ST}$ 's that ranged from .349 (in the genus Partula) to .777 (in Asterias). Between subspecies of Drosophila willistoni (D. willistoni and D.w. catum), a  $\bar{G}_{ST}$  of .192 was obtained. A variety of results were obtained from intraspecific comparisons. Eight subpopulations of Theba distributed over 100 miles had a  $\bar{G}_{ST}$  of .236. The  $\bar{G}_{ST}$  for five populations of Drosophila equinoxialis was .083. The Yanomama Indian villages, that are considered subdivided on the basis of demographic history (Neel and Ward, 1972) show a  $\bar{G}_{ST}$  of .066.

Most of these studies were based on a relatively large number of polymorphic loci; however,  $\bar{G}_{ST}$ 's obtained from a low number of polymorphic loci as in this study, and those calculated from a large number of polymorphic loci do not differ appreciably in their ability to detect differentiation (Fuerst, personal communication). The  $\bar{G}_{ST}$  of .156 obtained in this study is sufficient to confirm population subdivision and to substantiate (along with the  $G_H$  values) the isolation of the closed populations.

Isolation of populations can be caused by several factors. In the island model (Wright, 1943) and the stepping stone model (Kimura and Weiss, 1964; Weiss and Kimura, 1965) subpopulations may be separated from each other by geographical barriers that interrupt panmixis and normal movement patterns (Wright, 1951). Similarly, when the geographic range of an organism is much greater than the vagility of an individual, colonies may become semi-isolated because of the distance between groups of organisms (i.e. isolation by distance) (Wright, 1943; Kimura and Weiss, 1964). Both the island and the stepping stone models predict that the migration rate (thus the degree of differentiation) should be correlated with the distance separating the populations (Jaenike, 1973). An estimate of distance between each pair of populations of P. pugio was made from a map of the Galveston area. This was measured as the distance via the closest



water path between populations, plus, for each closed population, a distance to the water by the shortest land route. These distances are listed in Table 9. A correlation analysis of distances between populations and the standard genetic distance between those two populations yielded a non-significant value. This indicates that for these populations, differentiation is not retarded by nearest neighbor migration.

Numerous studies have documented population subdivision in natural populations. However, there have been some differences regarding how much differentiation is due to different selection pressures or random drift. Selander and Kaufman (1975) calculated  $F_{ST}$ 's from electrophoretic data on colonies of Helix aspersa located on two adjacent city blocks in Bryan, Texas. Not only were large shifts in allele frequencies found between the blocks but also among colonies within a block. The mean  $F_{ST}$ 's for the two blocks were .027 and .041; the mean  $F_{ST}$  for both was .034. The heterogeneous nature of blocks and colonies reflects reduced migration among colonies. There were no discernable habitat differences that corresponded to the pattern of variation observed. Because of this and small colony size ( $\bar{N}=15$ ), it was concluded that the intercolony differences were primarily due to random genetic drift. The same conclusion was drawn concerning the intercolony heterogeneity of

Table 9

Distance Estimates between Experimental  
Populations of P. pugio (in miles)

	LagP	Lag	Cem	SB	SAr	SLP	SR	WB
Lag	0.1							
Cem	15.1	15.0						
SB	13.5	13.4	6.5					
SAr	14.3	14.2	7.3	0.8				
SLP	13.9	13.8	7.2	5.2	6.0			
SR	13.2	13.1	6.5	4.0	4.8	3.7		
WB	14.2	14.1	7.5	5.0	5.8	4.7	1.0	
GL	17.7	17.6	11.5	9.0	9.8	9.5	5.1	5.2

Helix in Europe (Selander and Kaufman, 1975). In agreement with these results, Halkka (1970) found evidence of drift in another invertebrate, the spittlebug Philaenus. (However, Halkka and Halkka (1974) found evidence of selection for color polymorphisms in Philaenus transferred to islands.) In this case, small island populations were determined to be living under similar selective pressures to the mainland population. Heterogeneity of color polymorphism was attributed to random genetic drift operating against appreciable selective pressures. Thus, invertebrates with low vagility and low  $N_e$ 's commonly show population differentiation.

A major problem encountered in most studies like the above is that it is difficult to determine presence and magnitude of selection on enzyme systems. The presence of selection pressures for human blood groups and serum proteins has not been established; therefore, any change in frequency of blood groups could be due to random causes (Lewontin, 1974). Studies of human isolates or subdivided populations have commonly uncovered divergent frequencies of blood group and serum protein alleles for which genetic drift is the most probable explanation (Glass, 1952; Neel and Ward, 1972). Workman and Niswander (1970) discovered significant differentiation among Papago Indian districts. These differences appeared to be the result of differences

in the ancestral populations with further differentiation by random genetic drift due to small populations (300-1,000 individuals) of highly endogamous individuals. However, it is difficult in any subdivided organization to attribute apparent random differences to either sampling errors at reproduction or founder effects in the establishment of new colonies.

The above studies of genetic differentiation document that subdivided population structure can be established by means other than local selection differences. The P. pugio populations were chosen to collect additional (and hopefully less ambiguous) evidence relating population size to subdivision and variability. To investigate the possible causes of subdivision in P. pugio (indicated by genic diversity) comparisons of collections must now be made based on differences in other population parameters.

Since it has been established that these populations are genetically differentiated, it seems opportune to discuss the associated effects on genic variability and to speculate on causal factors. The values for the three common measures of genetic variability (i.e. percent polymorphism ( $\bar{P}$ ); number of alleles/population ( $n$ ); and average heterozygosity ( $\bar{H}$ ) are consistently lower for the small isolated populations of P. pugio. There is a loss of rare alleles in the closed populations which aids in the reduction of all three

measures. At the Pgm locus, there is a trend of increasing frequency of the  $F_2$  allele, with M being replaced by  $F_2$  as the common allele in the smallest population, LagP. The LagP population is not only the most deviant in gene frequencies (see Table 6), but is lowest in all three variability estimates.  $\bar{P}$  is reduced by 50% in LagP, and by 25% in the next two smallest populations, Cem and SAR. The largest closed population, SLP, has higher values than the other three isolated populations for all three variability measures. Because of the substantial increase in total area, the SLP population appears to be intermediate in population size and would be expected to be intermediate between the two groups in variability. This is the case for  $\bar{H}$  ( $\bar{H}_{\text{closed}} < \bar{H}_{\text{SLP}} < \bar{H}_{\text{open}}$ ), and for number of alleles at polymorphic loci in a population ( $n_{\text{closed}} < n_{\text{SLP}} \leq n_{\text{open}}$ ).  $\bar{H}$  and number of alleles at polymorphic loci/population were both tested for correlation with area of the collection sites. A rough estimate of the area of the grassbed habitat on the south bank of West Bay ( $A=93,750 \text{ m}^2$ ) was used to compare to the average values for all open populations ( $\bar{H}_{\text{op}}=.779$ ,  $\bar{n}_{\text{op}}=17.8$ ). The correlation coefficient for  $\bar{H}$  with the area is .926 ( $P>.05$ ), and for number of alleles with area is .921 ( $P>.05$ ). Even though the  $r$  values are very high, a test with only five data points (and only two data points are reversed, Cem and SAR) makes it difficult to obtain a

significant value. In addition, population size and habitat may not show an exact correlation. Rough density estimates were made in LagP and Cem (a 15 m<sup>2</sup> area was sampled three times with a seine) giving 12.6 and 37.3 P. pugio per pull. Density in SAR was too low to sample in this manner; an area of approximately 60 m<sup>2</sup> was sampled repeatedly, yeilding a total of 48 animals. The bay did show a higher density than any pond. Three 5m<sup>2</sup> sweeps with a pushnet gave averages of 26.3 and 34.3 P. pugio in WB and GL, respectively. Taking into account the possibility of reversed data points in the correlation of habitat size and population size, the implication of the high r values is that the level of variability is related to habitat size.

Assuming that the population is arranged according to the island model with no migration between subpopulations, the random drift hypothesis predicts that changes in gene frequency in small populations should be random in direction, deviating from both the source population and other small populations in a random manner (Crow and Kimura, 1970). The above discussion supports the fit of the P. pugio populations to the island model; hence, each closed population should randomly deviate from all other populations. The measures assessing gene diversity between pairs of populations (i.e. genetic distance and correlation of homozygosities) show that the closed populations do not deviate from the other

closed as much as they deviate from the open populations; but the average genetic distance for pair wise comparisons between each closed population and the other closed populations is tenfold greater than the average genetic distance for open populations compared with other open populations. The  $G_H$  values reinforce this observation: that closed populations are more like other closed populations than open populations but do not show as much similarity to each other as the open populations show as a group.

A dendrogram was constructed using Nei's standard D's (Figure 2). The smallest distance is seen between the two closed populations, Cem and SAr. All of the open populations, with the inclusion of SLP, are more closely related than LagP is to any other population or than the other two closed populations are to any open population or SLP. LagP may be so divergent because it is the smallest population or the most isolated, being on the far eastern end of the island. The genetic distance between SLP and SB is unexpectedly small. Although SB is connected to West Bay and classified as open, it is possible that migration is restricted through the narrow opening connecting the two bodies of water. This would mean that SB and SLP are closed populations of intermediate size. The similarity in gene frequencies between these two may be due to a size similarity or an increased level of migration between them because of their

FIGURE 2

Dendrogram based on standard genetic distances  
(D, Nei) between subpopulations of P. pugio. .



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Among Populations

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LagP

Cem

SAr

SLP

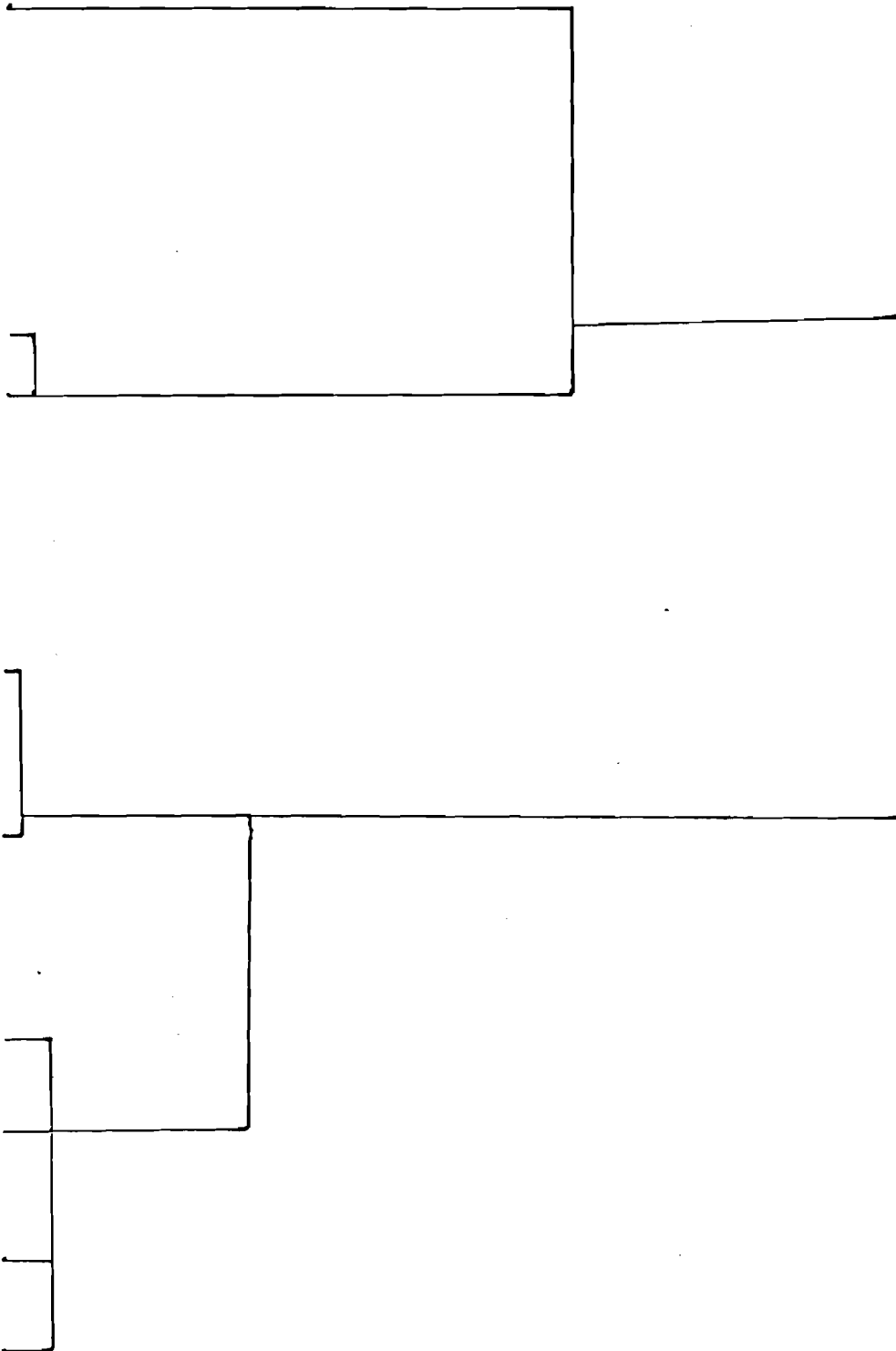
SB

SR

Lag

GL

WB



proximity; this end of the island is subject to flooding more than the east end.

The correspondence among the three smallest populations may be due in part to the small number and weakness of the polymorphism at the loci used in the study. However, it is suspicious that the Nada locus, which displays two alleles of intermediate frequency in the open populations, is fixed for the same allele in the three smallest populations and is tending in that direction in the largest closed population, SLP. This observation, as well as the overall genetic conformity of the closed populations, may be a reflection of the gene frequencies of the source population at the time of the founding event. The last time Galveston Island was completely flooded, connecting all of these bodies of water, was seventeen years ago during Hurricane Carla. This probably was the last time the isolated populations were connected by water to other populations. However, the source and age of the founding populations are not determinable. They may have originated from the bay population or from an already semi-isolated population that was not representative of the bay population (e.g. SLP may have been founded from the SB population, thus explaining this similarity). It is also possible that the founding event occurred much further back than seventeen years. The divergent character of the closed from the open may be partly explained then by different gene

frequencies in the ancestral population (founding effect) followed by drifting gene frequencies in the small populations. Further divergence of the open populations from the closed may be associated with the mechanisms for production and maintenance of genetic material in a population. Kimura (1968) and Wright (1951) predict that the effective number of alleles and genetic variability will increase in a subdivided population with high migration rates. Furthermore, a novel mutation (if not harmful) has a higher probability of survival in a large population (Kimura, 1962) and of moving to a higher frequency via migration in this type of population structure (Kimura, 1968; Kimura and Crow, 1964). This could explain the larger genetic distances between the closed and all the open, and between the closed populations with the other closed populations. The decrease in the number of rare alleles observed in the closed populations may be the result of the effects of any or all of the following causes: the founding bottleneck, genetic drift, and a reduced mutational load due to a small  $N_e$  (Nei, 1975; Kimura, 1968).

The similarities among the closed populations may be due to selection pressures that are comparable in this habitat type but different than pressures existing in the bays. Any type of diversifying or overdominance model must

be excluded because both are predicted to increase heterozygosity (Dobzhansky, 1970) and, therefore, would not account for the overall reduction in heterozygosity and variability seen in the closed population. However, some or all of the results could be due to strong directional selection for a particular allele(s) or genotype(s) at one or more loci, and this datum could be a direct consequence of that and/or some linkage effect. If directional selection pressures are present, there must be some environmental or ecological difference between the habitats encountered in the open versus the closed populations. The most obvious physical difference between these two systems is salinity. The closed ponds have a lower average salinity. However, LagP and SLP recorded the highest salinities, but deviate more from each other than from the other two closed populations. SLP shows the largest fluctuation in salinity, which is expected, because it is the shallowest and most subject to salinity rises in dry seasons or drops with high rainfall. There appears then to be no correlation between any allele frequency or variability measure and salinity. The same pattern of differences is probably true for temperature (and corresponding level of oxygen retention in the water) since physical buffering capacity against changes in these physical characters is a function of the depth and volume of water. Temperatures are likely to be more extreme in

the ponds than in the bay (due to the differences in volumes and depths of water), but Galveston Bay is also shallow and thus probably adjusts to air temperature changes fairly rapidly.

Studies have uncovered reduced variability connected with small  $N_e$ , but there are difficulties in concluding that drift, or selection, or a combination of the two is the causal factor. Perhaps the most drastic case of reduction in variability was found in the elephant seal, Mirounga angustirostris, by Bonnel and Selander (1974). Overhunting in the late 1800's ended in a bottleneck of less than 100 individuals. Although numbers have grown in excess of 30,000 animals, proteins encoded by 24 loci revealed no variability. In another study, Avise and Selander (1972) found reduced variability in small, partially isolated populations of cave dwelling Astyanax and attributed this primarily to genetic drift, although selection pressures could not be completely ruled out.

It is difficult to sort out the relative effects of selection, migration, and drift, when looking at subdivided populations; thus, much of the data showing reduced variability can be interpreted as a result of any one or a combination of these factors. Ayala et al. (1971) examined four island and six mainland populations of Drosophila willistoni. Only a slight reduction in  $\bar{H}$  was found on

island populations (16.9%, to 18.4% on the mainland), and in  $\bar{P}$  (79.5% on islands to 82.4% on the mainland). They make the claim that enzyme polymorphisms are remarkably similar in all the populations, but show no statistical tests to support this. Based on data gathered in the 1950's on the occurrence of chromosomal polymorphisms, the authors conclude that the island populations were established by small numbers of founders and are now geographically isolated, ruling out migration as responsible for observed similarities in allozyme frequencies and attributing the observed similarities to balancing selection (Ayala et al., 1971). However, it is at best difficult to draw these conclusions based on two sets of data collected twenty years apart, neither having been subjected to statistical analysis. Patterns of migration may easily have changed in the West Indies with the increase in boat traffic over the last twenty years. Until some selective pressures can be shown and migration more convincingly ruled out, it appears this population homogeneity may be maintained by migration.

In an attempt to find a more reliable way of detecting natural selection, Lewontin and Krakauer (1973) designed a test for the homogeneity of the inbreeding coefficients ( $F_{ST}$ , or the effective inbreeding coefficient,  $F_e$ ) for all the alleles at polymorphic loci in a group of populations. This assumes that the populations were founded from the

same parental population at the same time. This test is based on the premise that natural selection affects each allele at a locus, as well as each locus, differently. In contrast, the effect of any component of the breeding structure (e.g, migration, inbreeding, population size) is the same for all alleles and loci, yielding  $F_{ST}$ 's similar for all alleles. Therefore, if the alleles under investigation are all neutral (frequencies are dictated by only breeding structure), the  $F_e$ 's will be homogeneous, whereas natural selection will produce heterogeneous  $F_e$  values. Thus, the presence of natural selection is implicated when the observed variance in  $F_e$ 's is significantly greater than the theoretical variance of the  $F_e$ 's that is expected when all alleles are neutral.

Applying this test to natural populations, Nevo et al. (1975) concluded from the results of the Lewontin-Krakauer test that the high heterozygosity values observed in eleven populations of Bufo viridis (five central populations, two marginal populations, and four isolates) were maintained by selection. Objections have arisen over the validity of the use of this test for natural populations. Nei and Maruyama (1975) and Robertson (1975) pointed out that the expected variance used by Lewontin and Krakauer is an underestimate of the actual theoretical variance (Nei and Maruyama performed Monte Carlo simulations supporting this

contention). A basic assumption for the test is that the  $F_e$ 's are equal for all loci under neutrality. However, when mutation and high rates or special patterns of migration occur, this requirement is not fulfilled (Nei and Maruyama, 1975). Lewontin and Krakauer (1975), in response to the above criticisms, proposed that the only population structure that might meet the migration requirements for applicability of the test is an island model with populations founded at the same time (see also Gillespie, 1976). This alteration still does not take into account the influence of unique mutations on  $F_e$ 's.

The populations of P. pugio have been shown to fit the island model. There is an apparent heterogeneity in the  $G_{ST}$ 's for the four polymorphic loci. I feel that this is due, in part, to lumping alleles at two loci (Pgi and Mpi, which have low  $G_{ST}$ 's compared to the high  $G_{ST}$ 's for the two loci with no lumping). Therefore, due to the weakness of the test and the loss of information because of this scoring technique, I feel the test is not useful for my data.

If the physical characteristics of a population are influential in determining the genetics of a population, similar trends should occur in similarly structured species. Since the B. viridis populations (Nevo et al., 1975) approximated the island model, the data is comparable with



my data for P. pugio, although it is interpreted differently. Even though the populations are not highly differentiated ( $\bar{G}_{ST}$  for eleven populations is .059, Fuerst, personal communication), there is a significant reduction in  $\bar{P}$  and  $\bar{H}$  in the isolates compared to the central populations. On the basis of the results of the Lewontin-Krakauer test, the genic similarity of isolated populations, and the presence of a cline in gene frequencies at two polymorphic loci, natural selection is concluded to be the factor responsible for population differentiation. The neutral hypothesis and random drift are rejected as responsible for any part of the observed genetic pattern because of genic similarities among populations, absence of alternative fixations in isolated populations, and high  $\bar{H}$  in one of the isolates. Having calculated  $\bar{H}$ ,  $\bar{P}$ , and  $n$  for each population, I have some doubt as to whether these populations are truly isolated. The isolate, Jericho, has a higher  $\bar{H}$  than some of the central populations. It also contains more alleles than all the populations except for one central one. For this to occur, this population must not have suffered any bottleneck at the time of isolation and must also independently maintain a large enough population size such that no alleles are lost at reproduction, or have strong selection for all rare alleles or heterotic genotypes (Dobzhansky, 1970). Perhaps a more plausible explanation for such a

highly variable gene pool in an isolate is migration from surrounding populations. (If this is the case for this one isolate, it may also be the case for the other isolates.) The lack of alternative fixations does not necessarily disprove either hypothesis. Alternative fixation of alleles is found in the only island population, but it was not used in some analyses because of small sample size ( $n=10$ ). This collection was eliminated from the argument disfavoring drift, but used to support the hypothesis that disruptive selection is responsible for some of the genetic patterns. In answer to criticisms of the Lewontin-Krakauer test, the dissimilarity of  $\bar{H}$  values for different classes of enzymes is used as an independent test establishing the presence of selection for maintaining high variabilities. However, this is predicted by the neutralist hypothesis also. Purifying selection for highly constrained enzyme configuration would eliminate almost all mutant enzymes, whereas the classes of enzymes with less constrained configurations would accumulate neutral mutations that do not affect their catalytic properties (from a selection standpoint) and would be subject to random processes. Therefore, Nevo's data does not prove either case. The data showing a cline with aridity for two loci would appear to be a result of selection. If natural selection is an important force in maintaining the high variability in B. viridis, this is

then an example of reduced variability in isolated populations in the face of selection for high variability in the species. The pattern of variability of B. viridis could be the result of an interaction of selection and small population size.

The same interaction of selection and effect of small population size or selection alone cannot be ruled out as the cause for the genetic patterns observed in P. pugio. Admittedly, the variability in the closed populations may be too consistently deviant in the same direction, particularly for the Nada locus. However, the probability of three out of four populations being fixed for the same allele at a particular locus (assuming the alleles were initially in equal frequencies) is 0.25. Therefore, the data from the Nada locus does not discount the neutralist-random drift hypothesis as the factor responsible for the results. In addition to the smaller size, these populations probably suffer a more severe bottleneck in the winter in the less buffered habitats with no place to escape. With little or no migration, variability would not be restored to previous levels.

These data appear to be a good fit for the random drift hypothesis. However, the similarities among the closed populations are greater than is expected under a strict stochastic model. The observed patterns in genetic variability

may be a result of the interaction of some selection and small population size. Results of this study indicate that population size is the primary factor related to the genetic trends observed in this study. Further sampling and analysis of the genetic data from these populations, as well as more extensive monitoring of physical features related to these populations, should answer some of the questions arising from this study. The population structure of Palaemonetes lends itself well to such a study, and further use of Palaemonetes as an experimental organism should prove beneficial in future studies in population genetic theory.

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