Biochemical Population Genetics and the Spiny Lobster Larval Recruitment Problem: an Update

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RESUMEN

En una reciente reunion del GCFI presentamos nuestros primeros resultados en el uso de marcadores (o índices) genéticos bioquímicos para distinguir varias poblaciones geográficas de la langosta espinosa, *Panulirus argus* (Menzies y Kerrigan, Proc. GCFI, 1979). Este enfoque depende, en parte, del empleo de localidades geneticamente polimórficas con suficiente variabilidad en la frecuencia de los aleles que permitan detección de diferenciación geográfica, si ella existe. Con el empleo de más localidades genéticas para comparación de las poblaciones entre sí, mayor será la confianza en reconocer diferenciaciones, o su inexistencia. En la reunión de Cancún presentamos datos sobre varias localidades polimórficas demostrando diferencias entre Florida y Belize, y las Islas Vírgenes y la Florida. Desde entonces hemos identificado 8 localidades polimórficas e investigado seis poblaciones escogidas: Cayo Elliott y Cayo Hueso, Florida; Cancún, México: St. Thomas, Islas Vírgenes; Kingston, Jamaica y Trinidad.

Comparaciones de localidades por localidades mostraron que casi toda la población era característica. Sin embargo, el empleo de propiedades aditivas de la estadística "G" (Sokal y Rolff, Biometría, W.D. Freeman, 1969) a través de todas las localidades permite una interpretación más conservadora: Jamaica y St. Thomas son características, o singulares, con respecto una a la otra, así como en las poblaciones de la Florida y Cancún. El reclutamiento post-larval entre esas islas con poblaciones continentales no parece probable. Sin embargo, parece existir una relación entre Cancún, Cayo Hueso y Cayo Elliŏtt. Estos datos no sostienen un patrón de reclutamiento Pan-Caribe. Aun que algun reclutamiento puede efectuarse a través de largas distancias, estos datos sugieren que existe un intercambio post-larval significante que puede ocurrir a distancias más limitadas.

La ausencia de diferencias entre Trinidad y Cayo Hueso es enigmática. Sin embargo, según sean examinadas más áreas y localidades, la posición de Trinidad, así como de otras localidades geográficas en lo que respecta al patrón de reclutamiento post-larval pudieran ser aclaradas.

INTRODUCTION

Several groups and agencies, both U.S. and international, have in recent years identified the spiny lobster larval recruitment problem as being one of the most critical to the development of sound management policy for the pan-Caribbean industry (Jones, 1975; IOCARIBE, 1977). The long (6-12 month) pelagic planktonic phase of the larvae called phyllosomes coupled with the water movement patterns of the region make it difficult to identify parental stocks for the fishery of any country or region. The principal hypothesis was that of foreign recruitment. This hypothesis asserts that the principal path of post-larvae destined to grow to adulthood in Florida is through the Caribbean by way of the Yucatan Channel. Parental stocks might accrue from the western Caribbean or even as far as the north coast of South America, for example, Brazil. This hypothesis was based largely on work done by scientists from the Florida Department of Natural Resources — notably Sims and Ingle (1967). Lyons (1981) has reviewed the subject

for these Proceedings. In summary these studies assess the density of various developmental stages of spiny lobster phyllosomes in the Florida straits, Gulf of Mexico and Yucatan Channel, and the behavior of drift bottles after release in the Yucatan Channel. Given the long planktonic phase of phyllosomes, originally estimated at 6 months (Lewis, 1951), and the known current patterns (Gordon, 1967; Murphy et al., 1975) it is not difficult to envision their being transported long distances.

Studies over the past decade on the currents of the Caribbean are consistent with the presumption of net east-to-west movement of water and larvae entrained in a time frame compatible with transport to Florida. However, many of these same studies have also revealed a water movement indicating long entrainment in the vicinity of certain islands (Molinari et al., 1980). Further, the existence of local currents and gyres flowing against the net east-west path have been revealed (Duncan et al., 1977; Metcalf and Stalcup, 1974; Metcalf et al., 1977; Grant and Wyatt, 1980). Similar currents exist in the region of the Florida Straits (Kielman and Duing, 1974; Brooks and Niiler, 1975; Lee and Mayer, 1977). These observations suggest that some phyllosomes might be entrained in the regions of their origin. The numbers need not be excessive to have a significant impact on local recruitment. If two larvae from each spawning female survive through maturity and spawn locally, steady state population numbers will be maintained. Thus larvae from non-local sources would not be needed to sustain the population. Even survivorship rates, from loss and local mortality, as high as 10^{-5} would still be compatible with this mechanism. The critical questions therefore are: do significant numbers of non-local well-travelled larvae settle in near-shore waters of Florida to begin their benthic existence, and if so do they have the necessary genetic components to survive?

Since most approaches (plankton and water current studies) used to distinguish between the two recruitment mechanisms are inferential, we proposed biochemical population genetics as an alternative approach (Menzies, Kerrigan and Kanciruk, 1978). If wide spread mixing of phyllosomes occurred coupled with long distance recruitment, one would expect *Panulirus argus* to be genetically very homogeneous throughout the range. On the other hand, if genetic differentiation existed, "long-distance" recruitment might not be so important. In our last paper (Menzies and Kerrigan, 1979) we reported preliminary evidence for genetic differentiation between three populations in at least one of three loci examined. Those results were consistent with the concept that recruitment was not pan-Caribbean. That is, there did appear to be boundaries beyond which larval exchange or transport was not significant to recruitment. Since that report we have continued our studies in an attempt to better determine what those boundaries are. We have concentrated on examining a few selected populations for as many genetic loci as possible. The populations were selected in part for their location within the range of *P. argus* and in part on their availability. This paper is an interim report of work still in progress. We have now examined eight polymorphic loci and have extended our observation of genetic differentiation among six selected populations.

METHODS AND MATERIALS

Animal Collection. Adult and subadult *P. argus* were obtained either by diving or purchase from fishermen. They were kept on ice until frozen and then transported to

Table 1. Summary of enzyme-loci

Enzymes†	E.C.‡	Loci§ Detected	Tissue(s)‖	Allele(s) Scored	
GPD	1.1.1.8	1	L*,An.,T,g.g.	None	Resolution insufficient for scoring.
LDH	1.1.1.27	LDH-1	L*,E,H,An.	3	Polymorphic, see table 2.
		LDH-2	T*,g.g.,An, E,H	1	Monomorphic for 3 population studied.
MDH	1.1.1.37	MDH-1	L*,T*,gill	None	Resolution insufficient for scoring, both Loci
		MDH-2	HP,g.g.,An.	None	are weakly polymorphic.
ME	1.1.1.40	1	L*,An.,T	1	Monomorphic for two population studied (120 animals).
ICD	1.1.1.42	1	L*,g.g.	None	Good resolution, polymorphic; but not used for Pop. study.
GD	1.1.1.49	1	T*,L,An.,g.g.	None	Resolution insufficient for scoring.
GAPDH	1.2.1.12	1	L*,An,T	None	Resolution insufficient for scoring.
GLUD	1.4.1.3	GluD-1	An*,L,T	None	Good resolution, Polymorphic; but not used in Pop survey.
		GluD-2	An*,L,T	1	Monomorphic for two population studied (126 animals).
GOT	2.6.1.1.	GOT-1	L*,An,g.g.	1	Monomorphic for two population studied. (141 animals).
		GOT-2	L*,An.g.g.	None	Resolution insufficient for scoring.
PGM	2.7.5.1.	1	L*,An,T,g.g.	3	Polymorphic, see table 2.
E	3.1.1.1.	EF-3	HP*,An,L,T	3	Polymorphic, see table 2.
E	3.1.1.8	EF-4	T*,L,An,HP	4	Polymorphic, see table 2.
UP	3.1.3.2.	UP-L	L*,T	4	Polymorphic, see table 2.
		UP-H	HP	None	Resolution insufficient for scoring.
U-GAL	3.2.1.22	1	L*,T	1	Monomorphic for four Population studied (180 animals).
PEP-B	3.4.13.11	PEP-B-1	HP*	2	Polymorphic, see table 2.
		PEP-B-2	HP*	3	Polymorphic, see table 2.
PEP	3.4.11.4	PEP-LLL-1	HP*	1	Monomorphic for all six population studied (286 animals).

Table 1. Continued

Enzymes†	E.C.‡	Loci§ Detected	Tissue(s)‖	Allele(s) Scored	
		PEP-LLL-4	HP*	1	Monomorphic for all six population studied (286 animals).
PEP-D	3.4.13.9	1	HP*	3	Polymorphic, see table 2.
GPI	5.3.1.7	1	L*,An,g,gg,T	None	Resolution insufficient for scoring.
LAP	3.4.11.1	1	L*,HP,g.g.,gill	1	Monomorphic for two population studied (114 animals).

†GPD, glycerol phosphate dehydrogenase; LDH, lactic dehydrogenase; MDH, Malate dehydrogenase; ME, malic enzyme; ICD, isocitrate dehydrogenase; GD, glucose 6 phosphate dehydrogenase; GAPDH, glyceraldehyde phosphate dehydrogenase; GLUD, glutamate dehyrogenase; GOT, glutamic-oxaloacetate transominase; PGM, phosphoglucomutase; E, esterase; UP, umbellyferyl phosphatase; U-gal, umbellyferyl galestosidase; PEP, peptidase; GPI, glucose phosphate isomerase; LAP, leucine amino peptidase.

‡Following Harris and Hopkinsons (1976) use of enzyme nomenclature as recommended by the Int. Union of Biochemistry.

§Loci numbered in order from anode.

‖L, Leg muscle; An, antenna muscle; T, tail muscle; g.g. green gland; H, heart; HP, Hepatopancreas.

the laboratory in Ft. Lauderdale, Florida.

Pueruli and post-larvae were collected with the use of Witham habitats (Witham et al., 1968; Sweat, 1968) similar to those modified and described by Little (Little, 1977; Little and Milano, 1980). These were deployed on both the ocean and bay-side of Elliott Key, Florida in Biscayne National Park. Monitoring was conducted on a weekly basis when possible with the aid of National Park personnel.

Tissue Preparation. Dissection, storage, and tissue homogenization procedures were identical to that previously reported (Menzies and Kerrigan, 1979) with one exception. Most tissues were homogenized in deionized water in a 1:2 or 1:1 (w/v) ratio.

Electrophoretic Analysis. Esterases were analyzed on polyacrylamide gels as previously described (Menzies and Kerrigan, 1979). Peptidase (pep B1, B2, D, LLL-1, LLL-4) were analyzed on 14% starch gel (Electrostarch Co., Madison, Wisc.), bridge buffer was 0.3M borate, pH 8.6 and gel buffer was 0.005M tris-citrate, 0.02M $MgCl_2$ at pH 7.5. The remaining enzymes were analyzed either with this buffer system or a gel buffer of 0.015M borate, pH 8.6 with 0.01M $MgCl_2$. See Table 1 for a list of the enzymes studied and their tissue of origin. Specific enzyme staining reactions were essentially the same as described by Harris and Hopkinson (1976). Substrates used for peptidase assays were leu-pro for Pep D, leu-gly for Pep B1 and B2 and leu-leu-leu for Pep LLL-1 and LLL-4. All enzyme staining reagents were obtained from Sigma Chemical Co., St. Louis, Mo. Scoring techniques were previously described (Menzies and Kerrigan, 1979). Loci and number of alleles scored are summarized in Table 1 and allele frequencies in Table 2.

Table 2. Polymorphic loci; genotype and allele frequencies

Enzyme/Population	Genotype (No. Animals)								Alleles (Fractional)			
	1/1	2/2	3/3	1/2	1/3	2/3	X/4	Total	1	2	3	4
LDH												
Elliott Key	0	37	3	0	0	10		50	0.0	0.84	0.16	
Key West	0	48	1	0	1	8		58	0.01	0.90	0.09	
Cancun	0	28	0	5	1	3		37	0.08	0.86	0.06	
Trinidad	0	26	0	0	0	3		29	0.0	0.95	0.05	
Jamaica	1	40	0	2	0	4		47	0.04	0.92	0.04	
Virgin Islands	0	17	2	0	0	18		37	0.0	0.70	0.30	
PGM												
Elliott Key	0	49	0	3	0	2		54	0.03	0.95	0.02	
Key West	0	37	0	9	0	0		46	0.1	0.9	0.0	
Cancun	0	28	0	4	0	0		32	0.06	0.94	0.0	
Trinidad	0	31	0	0	0	0		31	0.0	1.0	0.0	
Jamaica	1	38	0	1	0	0		40	0.04	0.96	0.0	
Virgin Islands	0	30	0	7	0	1		38	0.09	0.89	0.01	
ESTERASE EF3												
Elliott Key	42	13	0	35	0	2		92	0.65	0.34	0.01	
Key West	8	17	0	29	0	4		58	0.39	0.58	0.03	
Cancun	1	15	0	14	0	6		36	0.22	0.70	0.08	
Trinidad	9	5	0	16	0	2		32	0.53	0.44	0.03	
Jamaica	19	4	0	17	0	11		51	0.54	0.35	0.11	
Virgin Islands	13	5	0	24	0	2		44	0.57	0.41	0.02	
ESTERASE EF4												
Elliott Key	19	20	0	36	3	14	2	94	0.41	0.49	0.09	0.01
Key West	14	5	0	17	1	3	0	40	0.58	0.38	0.04	0.0
Cancun	10	7	0	14	1	4	0	36	0.49	0.44	0.07	0.0
Trinidad	7	14	0	11	0	0	1	33	0.38	0.61	0.0	0.01
Jamaica	14	12	0	18	2	5	0	51	0.47	0.46	0.07	0.0
Virgin Islands	7	11	2	13	3	3	0	39	0.38	0.49	0.13	0.0

Table 2. Continued

Enzyme/Population	Genotype (No. Animals) 1/1	2/2	3/3	1/2	1/3	2/3	X/4	Total	Alleles (Fractional) 1	2	3	4
PEPTIDASE B1												
Elliott Key	44	0		22				66	0.83	0.17		
Key West	27	1		15				43	0.80	0.20		
Cancun	12	0		19				31	0.69	0.31		
Trinidad	24	0		4				28	0.93	0.07		
Jamaica	3	3		14				20	0.50	0.50		
Virgin Islands	22	0		0				22	1.0	0.0		
PEPTIDASE B2												
Elliott Key	0	46	0	3	0	9		58	0.03	0.90	0.07	
Key West	0	22	0	1	0	20		43	0.01	0.76	0.23	
Cancun	0	15	0	4	0	10		29	0.07	0.76	0.17	
Trinidad	0	8	2	0	0	15		25	0.0	0.62	0.38	
Jamaica	0	8	0	0	0	36		44	0.0	0.59	0.41	
Virgin Islands	0	26	0	0	1	3		30	0.02	0.92	0.06	
PIPTIDASE D												
Elliott Key	0	27	25	4	1	40		97	0.03	0.50	0.47	
Key West	0	19	16	1	1	28		65	0.02	0.52	0.46	
Cancun	0	15	5	1	0	17		38	0.01	0.63	0.36	
Trinidad	0	8	3	4	2	14		31	0.10	0.55	0.35	
Jamaica	0	23	10	3	0	14		50	0.03	0.63	0.34	
Virgin Islands	0	17	10	0	0	18		45	0.0	0.58	0.42	
UMB-PHOSPHATASE												
Elliott Key	1	2	34	1	6	19	1	64	0.07	0.19	0.73	0.01
Key West	1	2	26	0	4	23	2	58	0.05	0.23	0.70	0.02
Cancun	5	1	19	2	4	6	0	37	0.22	0.14	0.64	0.0
Trinidad	0	0	18	1	1	12	0	32	0.03	0.20	0.77	0.0
Jamaica	7	2	19	1	2	16	1	48	0.18	0.22	0.59	0.01
Virgin Islands	0	0	31	0	4	0	4	39	0.05	0.0	0.90	0.05

Statistical Analysis. A variety of approaches to data analysis have been tried but most germaine to the study is the "G" statistic described by Sokal and Rohlf (1969). While traditional Chi-Square methods will allow locus-by-locus tests of homogeneity of genotype frequencies between pairs of populations, results for each locus are not additive. For a variety of reasons this could be misleading in determining the genetic relatedness between populations. However, the G statistic performs the same analysis on a locus-by-locus basis and because of its additive property allows an "averaging" over all loci studied simultaneously. To further the conservative nature of the statistic, category frequencies (animal numbers in a genotype group) were normalized to numbers of the smaller of two populations compared. The choice of genotype frequencies rather than allele frequencies for comparison between populations was based on the fact that in diploid organisms it is the genotype that is the unit of evolution or survival (Hedrick, 1971).

RESULTS

Because of the labor intensiveness of this project, the following plan was adopted to maximize the rate of production of "fisheries management" useful information. The first goal was to identify as many polymorphic loci as possible. The second was to survey their allele frequencies in six selected populations. When this was complete, additional populations and loci could be added to the study.

Nineteen different isoenzyme systems representing 26 different loci were studied. Ten of the loci were not used in this study because of technical difficulties or failure to complete population analyses by this writing. Eight loci were found to be monomorphic after studying over 100 animals from two or more populations. Since monomorphic loci contribute little to an understanding of genetic differences between populations, their study was discontinued at this point. Eight loci found to be polymorphic were surveyed in the six selected populations. Details concerning the genetics and biochemistry of all loci studied will appear in other publications. Table 1 summarizes all loci studied.

For polymorphic loci, presumed genotype frequencies in terms of the number of animals observed in each class and allele frequencies for each locus and population are summarized in Table 2.

The G statistic was calculated for every pair of populations over each polymorphic locus. These were summed along with their degrees of freedom according to Sokal and Rohlf (1969). The calculations are summarized in a contingency table (Table 3). Significantly different pairs ($p<0.05$ are indicated by a star. Most noteworthy is the absolute uniqueness of the Jamaican and Virgin Island populations. These data are consistent with the supposition that neither of these populations receives nor contributes genes (and therefore larvae) to any of the other five populations. Elliott Key and Key West populations were not observed to be significantly different from each other, or different from the Trinidad population. On the other hand, certain intermediate populations, for example, Cancun and Jamaica, were different from both Elliott Key and Trinidad populations. Lack of difference in the case of Trinidad, Elliott Key and Key West could be real or an artifact of the calculations. The sample size for the Trinidad population was low (33 animals) and the G statistic, used here on a very conservative basis, could obscure real differences.

Table 3. "G" all loci

POPS	E.K.	K.W.	CAN.	TDAD	JAM.
K.W.	36.98				
CAN.	49.46*	24.20			
TDAD	30.47	28.18	54.08*		
JAM.	78.71*	66.03*	48.74*	36.64*	
V.I.	40.87*	63.42*	79.22*	54.89*	119.77*

*$p<.05$

Post Larval Collections. Although genetic studies on post larvae have not been completed, data on seasonality of influx to Elliott Key affect the recruitment question. A minimum of 20 and a maximum of 40 Witham habitats were monitored once a week for 14 months with one 4-week lapse in December 1978 and January 1979 due to equipment failure. Figure 1 is a graph of catch per unit effort as a function of time. Catch per unit effort refers to total post larvae captured per week including transparent pueruli, slightly pigmented and dark pigmented stages per habitat deployed. This graph illustrates the following: (1) Post larvae enter South Florida coastal waters (Elliott Key) all year; (2) A peak influx appears to occur in spring while minimum influx occurs in fall and early winter; and (3) The influx follows a monthly periodicity.

DISCUSSION

Comparison of populations of animals by genetic techniques depends on how well frequencies of genotype subgroups are determined and whether differences (not similarities) between subgroups can be detected. A few differences would be of greater significance in population comparison than a comparable number of similarities. Similarity does not prove identity. However, a conclusion of similarity would be strengthened by accumulative data, that is, a lack of differences over many genetic loci.

Determination of genotype frequencies depends in part on how accurately a genotype can be scored and partly on the population and genotype subgroup sample size. Ideally, one should have a sample size of 50 animals or more and comparable genotype groups should consist of 5 or more. In this report it was not always possible to meet those criteria since animal collection numbers ranged from 33 (Trinidad) to 99 (Elliott Key). Furthermore, genotypic determinations for every animal at every locus have not been completed. Nevertheless, application of corrections for small sample size (Sokal and Rohlf, 1969) have not appreciably altered the outcome of either Chi-Square or G-statistic tests.

The accuracy of scoring can also introduce error. In these studies genotype determination for each locus and animal was verified at least once. Each electrophoretic run consisted of animals from several populations randomly distributed on each gel. Except for standard animals, population identity of each

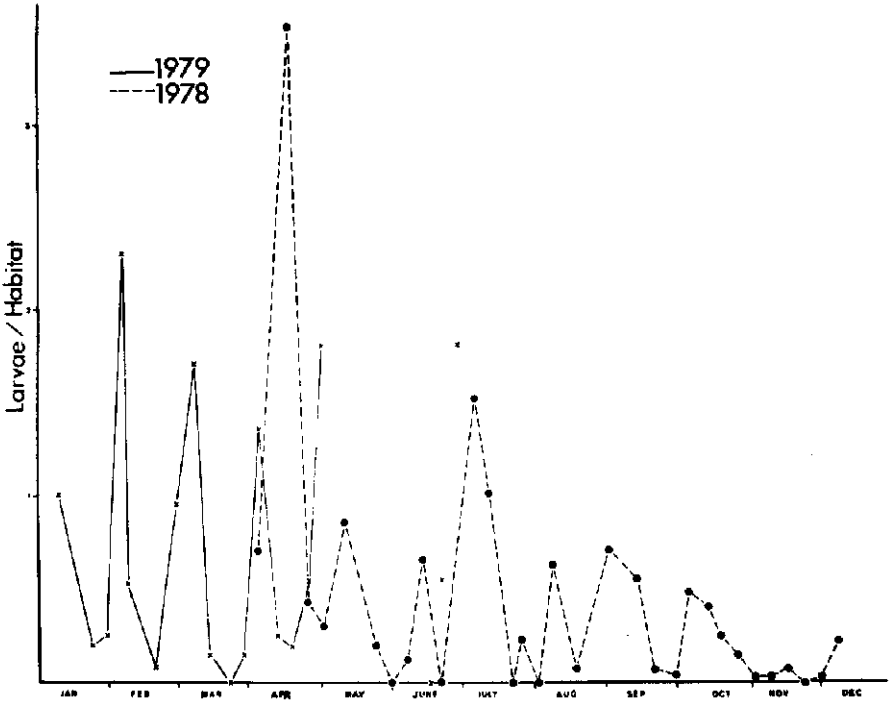


Figure 1. Post-larval (transparent and pigmented) influx at Elliott Key, Florida. Each point represents total post-larvae per habitat examined at weekly intervals.

score was unknown at the time of scoring. In most cases scoring was not difficult. However, Pep B1 was problematical because of variation in the intensity of color development. This explains the fewer accepted scores in several populations (Table 2).

Comparison of paired populations on a locus by locus basis by the G statistic revealed that each population was significantly different from most other populations for at least one locus. However, summing G over all eight loci allowed comparisons of the composite genotype. This is in effect an average over the loci and tends to minimize questionable differences. Table 3 is a contingency table of pair-wise sums of G. The starred values are significant at the 0.05 level. However all starred population pairs except Jamaica vs Trinidad are significant to at least the 0.01 level.

To interpret these results in terms of lobster larval recruitment, arrows have been drawn on a map of the Caribbean connecting all six populations by either the shortest or the most reasonable water routes that larvae might be expected to traverse (Fig. 2). If all pair-wise combinations that showed significant differences (Table 3) are removed from the map the result is shown in Figure 3. Thus, it appears that substantial gene flow does not exist between eastern, central, and western Caribbean locations. One exception — a lack of difference between Cancun and

Figure 2. (Left) Map of Caribbean showing routes between the six populations studied.
Figure 3. (Right) Map of Caribbean showing those routes not eliminated by genetic differences.

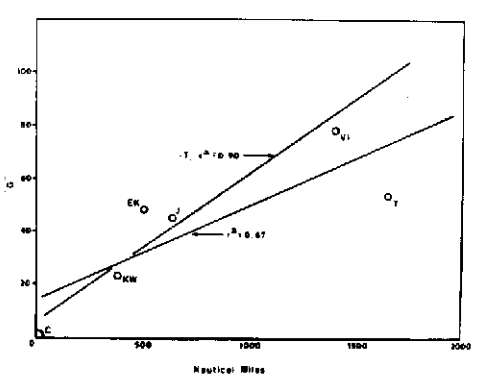
Key West — is consistent with possible larval input from the Yucatan area to the lower Florida Keys. Interestingly, Trinidad was not different from either Key West or Elliott Key, Florida. This possibly is an artifact of the small sample size. If the lack of difference is real it may be reflecting similar selective factors on the loci studied. However, it is difficult to imagine how Trinidad genes and larvae could be mixing with South Florida populations but not Jamaica, Virgin Islands or Yucatan populations.

The data in Table 3 demonstrate that genetic differentiation does exist throughout the range of *P. argus*. Therefore either geographic barriers have been sufficient to allow genetic drift to establish differentiation and/or ecological differences among Caribbean locations are such that local selection pressures establish and maintain genetic differentiation. If genetic drift, for example, random long-term established differences in allele frequencies, is the sole explanation then geographic isolation must be strong enough to prevent gene flow. Even a few percent input of larvae from initially distinct populations would erase genetic differences between those populations in only a few generations.

To illustrate this an estimate of migration rates can be obtained from the following equation (Nei, 1975):

$$I = (m_1 + m_2) / (m_1 + m_2 + 2\nu)$$

Here, m_1 and m_2 are the migration rates between populations 1 and 2, ν is the mutation rate per locus per generation and I is the normalized steady state genetic identity between the two populations. "I" is calculated from allele frequencies. Values we have obtained range from 0.88 to 0.98 for comparisons between the six populations. If we assume a value of 10^{-6} for mutation rate and m_2=o, for example, migration is unidirectional in an area such as South Florida, we obtain migration rates ranging from 10^{-4} to 10^{-5}. Thus, in the absence of natural selection, no more than one in ten thousand to one hundred thousand recruits per generation could come from genetically distinct populations without eliminating the existing genetic differences. Considering the nature of water transport patterns from the Lesser



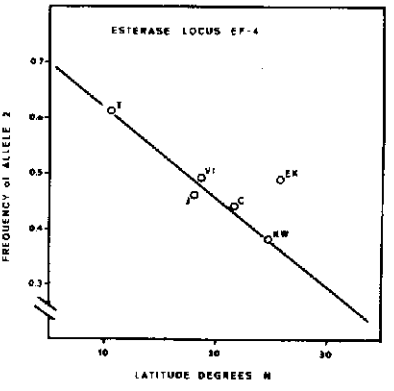


Figure 4. (Left) Relationship of G with distance from Cancun.
Figure 5. (Right) Relationship of the 2 allele of esterase EF-4 with latitude.

Antilles to Florida, local gyres and eddys not withstanding, it is difficult to imagine absolutely no larval transport between some of the populations studied. However, if response to local ecological factors, for example, natural selection, is significant, then geographical isolation need not be as rigorous. Migration rates might be greater than 10^{-4} - 10^{-5} but only compatible genotypes survive.

Several lines of evidence are consistant with the existance of significant selective forces. Allele frequency clines or a linear relationship between the magnitude of G and distance between pairs of population would not be expected in the case of genetic drift unless all populations compared were related by a common path. Instead, a geographically random array would be expected. To examine this, G values were plotted as a function of distance from a reference population and regression analysis performed. The reference population had a real value of zero; G values of the other five populations were plotted as a function of distance from this point. With few exceptions the results were similar for each chosen reference population. Five out of six populations showed a linear relationship of G with distance. The best example of this is shown in Figure 4 where Cancun, the western most population, was used as an anchor point. Whether Trinidad is present or deleted the regression coefficient is still significant, that is 0.67 and 0.90. Thus genetic difference appears to be maximized by distance and is not random.

The question of allele frequency clines was also examined. Although the numbers of populations and their longitudinal or latitudinal distribution in the study thus far are insufficient to adequately test this hypothesis, some clinal effects were observed. For example, in Figure 5, the 2 allele frequencies of Esterase locus EF-4 when plotted as a function of latitude show a highly significant regression. The line shown and regression coefficient of 0.97 were calculated after deleting the Elliott Key population.

In both examples above genetic drift could yield the same results only if all six populations were connected by a single path. There is no single reasonable water current path that connects all six populations.

Another line of evidence comes from lack of confirmation to Hardy-Weinberg

expectations of genotype frequencies of three loci in the Jamaica population. Esterase EF3 and phosphatase, UP, both showed homozygous excess whereas peptidase B2 showed heterozygous excess. In the former two cases there are a variety of mechanisms exclusive of selection which could give rise to the observed genotype frequencies. However, there are no reasonable explanations exclusive of selection for the latter case.

These data although too preliminary to prove the existence of natural selection of all loci are strongly suggestive for selection of some. Nevertheless, existance of genetic differentiation must be explained by some mechanism. A hypothesis of strictly local recruitment (absolute geographic isolation) is challenged by post-larval influx data. Figure 1 shows that post-larvae enter inshore waters year round although there are monthly and seasonal peaks. This has also been reported by Little (1977) and Little and Milano (1980). Further, Lyons (1981) has pointed out that lobster spawning in the Florida Keys (although occurring between April and September) peaks between May and June. If the planktonic period from first stage phyllosome to puerulus is relatively constant (5-7 months), as suggested by Lewis (1951), and if recruitment is totally from local stocks, influx of pueruli should increase in September, peak from October through December with virtually no pueruli coming in from late January through July. Our data and those of Little and Milano (1980) show a spring-summer peak from March through May, another smaller peak in June and post-larvae coming in year round. If the peak of post-larval influx consists mainly of locally spawned phyllosomes, then a planktonic period of 10 to 11 months would be more consistent with these data. Sims and Ingle (1967) had earlier concluded that the planktonic period was in excess of 6 months. A period of 10 to 11 months is similar to that estimated for the planktonic phase of other members of the genus *Panulirus*.

For yearly recruitment of post-larvae, there are two prominent explanations: (1) delayed developmental rates and (2) foreign recruitment, or at least arrivals. Since the former has never been observed for any related species, for example, superfamily Scyllaridea, the latter is the more appealing explanation. A rough estimate from our data (Fig. 1) and those of Little and Milano (1980) places the level of foreign arrivals in excess of 25%. If this is true and the observed genetic differentiation is confirmed with future analyses, two questions arise: (1) where are the post-larvae coming from and (2) what percent are surviving? We hope to answer these questions by comparing the genetic structure of different arrival groups of post-larvae and further comparing them to various adult populations. If post-larvae collections throughout the year do not differ from each other or from Florida adult populations, the foreign contingent must arise from a population whose genetic structure is similar to that of Florida's and has a broader spawning season. If we find genetic differences between post-larval collections throughout the year, especially if fall and winter post-larvae are different from Florida adults, taking into account the results of the above studies, it is unlikely that many of these post-larvae survive to maturity. The existence of local selection would effectively eliminate those post-larvae from becoming juvenile recruits to the Florida fishery.

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