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The Genetic Structure of Mussel Populations in Eastern Canadian Waters

by

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A paper on genetics of the edible or blue mussel, Mytilus edulis, must appear as somewhat of an anomaly in a symposium on stock discrimination in fishes and squid. However, during the past few years we have begun to understand certain features of both the genetic structure of mussel populations in local waters, and the relationship between this genetic structure and differences in important production parameters. It is thus, as an analogue for studies of differences in fish population productivity and stock structure, that mussel genetics has relevance.

Genetics of the Mussel Populations

Our studies of the genetic constitution of mussel populations of the Maritime Provinces may be divided into two categories, one macrogeographic and one microgeographic. Sampling locations are illustrated in Figure 1. In the macrogeographic part of the study, mussels were sampled from near or below the low-water level in four localities. Two of these localities were on the Atlantic Coast of Nova Scotia near Halifax (Bedford Basin (BB) and St. Margaret's Bay (SMB), later referred to as Luke Island (LI)). Two localities were in northern bays, one near the head of the Bay of Fundy (BF) and one in the southern Gulf of St. Lawrence at Ellerslie, P.E.I. (PEI). The sites were chosen to provide a contrast between the relatively stable environments along the Atlantic Coast and the more variable and seasonally extreme environments of the Bay of Fundy and Gulf regions.

In the microgeographic part of the study we sampled at six sites within St. Margaret's Bay. One of them (LI) was the reference station of

Part 1, to which we added two other "open-water" stations (Shut-in Island (SII) and North-west Cove (NWC)) and three "head-water" stations (Potatoe Island (PI), Todd Island (TI) and Frenchman's Point (FP)). In this case, we considered that the two additional open-water stations provided other representations of the relatively stable oceanic environment. The head-water stations were, however, near extensive tidal flats and are in regions of short-term and seasonal extremes of both temperature and salinity conditions. We considered that the head-water sites were variable environments comparable to the BF and PEI sites in the macrogeographic sampling.

To characterize the genetic constitutions of these stocks we adopted the isoenzyme technique. Horizontal starch gel electrophoresis, using the discontinuous Paulik buffer system of Singh and Zouros (1978), was carried out on samples of 100 animals chosen from collections of between 200 and 300 medium-sized mussels taken from the various sites. After preliminary experiments, we verified our ability to record multiple alleles at four gene loci for the macrogeographic collections (Gartner-Kepkay et al., 1980). The four loci were:

LAP-1 (leucine animopeptidase)
PEP-2 (peptidase 2)
PGI (phosphoglucose isomerase)
PGM (phosphoglucose mutase)

Subsequently, in time for the microgeographic study, we were able to add a fifth locus: AP (aminopeptidase) (Gartner-Kepkay et al., in press). At each gene locus between three and six alleles were identified as common to all the populations sampled.

The results can be illustrated by the data for the two gene loci, LAP-1 and PGM. In Figure 2A are two graphs of allele frequencies for LAP-1 obtained in the microgeographic and the macrogeographic collections. In the samples taken within SMB, it is clear that open-water sites show approximately the same frequencies of the three alleles that were recorded. By contrast, the head-water or variable environments all show a high proportion of the faster-migrating alleles and a low proportion of the slow-migrating alleles. A similar phenomenon is shown by the macrogeographic study. Here the Bedford Basin sample is most like the SMB sample. The PEI sample appears like the head-water samples and BF is intermediate. In fact, study of the other alleles recorded suggests that we might properly regard PEI-BF as one pair and SMB-BB as another, even if in this instance the two pairs were close to overlap.

A similar result may be seen in data for the PGM locus. In this case, there were six alleles common to the nine sampling sites. In the two panels of Figure 2B, the sampling within St. Margaret's Bay again shows the differentiation into head-water and open-water sites, while the macrogeographic collections differentiate the Atlantic Coast sites from the variable PEI-BF sites.

Results for the other two isoenzymes were similar for the macrogeographic study, although the separation by environmental types was not so clear for the PGI data within St. Margaret's Bay. Statistical comparisons among successive pairs of samples taken from the nine populations all reinforced the conclusions that these allele frequencies represented two population types. Accordingly, we have calculated indices of identity between sample locations based on the data for the four isoenzymes (Nei, 1972). The results are summarized in the dendrogram of Figure 3, which shows that the isoenzyme frequencies indicate close relationships for localities with the "fluctuating" environments and the "stable" environments, but much weaker relationships between these two environmental types.

By contrast with this generalization, the results obtained from the additional gene locus, AP (aminopeptidase), in the microgeographic study show no differences between localities (Fig. 3). Four alleles were identified at this locus in populations at all the sampling localities within St. Margaret's Bay, but only one locality showed a slight deviation from an apparently common frequency distribution among localities. This observation is of significance in interpreting our over-all results.

One further set of data is useful in interpreting this evidence of genetic population segregation in the mussel. It is recognized from isoenzyme studies that among mollusc populations generally, the allele frequencies tend to show a deficiency in the relative occurrence of heterozygotic animals in the population, compared with the proportions that would be expected at genetic equilibrium (Levinton and Koehn, 1976). This characteristic was also evident in the samples we recorded. We have therefore calculated the heterozygote deficiency values for each isoenzyme at each sampling location. The results for the five isoenzymes recorded for the six sampling localities within St. Margaret's Bay are shown in Table 1, as the percentage decrease in observed heterozygote frequency compared with the expected frequency at Hardy-Winberg equilibrium. Frequency deficiencies range from 30% to 70% for the four gene loci which showed microhabitat separation, but are distinctly less for the

AP locus. There are strong statistically different proportions of heterozygotes among the gene loci within each sampling locality. There were also distinctly different proportions of heterozygotes among localities for the LAP-1 and PEP-2 loci.

Conclusions from the Isoenzyme Study

The results show that there are distinct differences in the populations of isoenzymes present in mussels at various localities in the Maritime Provinces. On the basis of macrogeographic data for the LAP-1 and PGI enzymes, one might also conclude that this isoenzyme difference indexed a difference in the populations of the mussels themselves. However, this interpretation is made suspect by the fact that the PEI-BF pair was apparently based on environmental similarity in the face of the widest geographical separation of sample locations. This relation of genetic structure to microhabitat characteristics is confirmed by the difference between localities within St. Margaret's Bay, since with the generally active water circulation and exchange within this Bay there is good reason to suppose that these microhabitat differences among adults are a result of selective survival from a more or less homogenous population of zygotes. This hypothesis is confirmed by the fact that the AP isoenzyme was uniformly distributed throughout the Bay and showed a near equilibrium population of alleles. The fact that the alleles showing differences among localities also showed strong heterozygote deficiency confirms that these distributions must have been subject to active selection pressure.

It is clear, then, that the isoenzyme population differences that we have observed cannot be held to represent stock divisions so much as they reflect microhabitat environmental differences. That is not to say that the differences in isoenzyme populations do not at the same time index significant population production differences. They may well do so. However, our evidence for differences among the assortment of the different isoenzymes suggests that the allele frequencies have been independently determined by different environmental selection forces. For example, the AP isoenzyme seems to be related to some basic characteristic of the species M. edulis, while the highly variable LAP-1 may well be associated with the physiological mechanisms that permit osmotic adaptation to environmental changes in salinity (Koehn, 1978). We do not know the physiological functions of the other enzymes, but evidently the job of determining whether any one or any

particular grouping of the isoenzymes may index a difference in productive capacity requires separate specific experiments. At present, we are now trying to develop these studies.

From these results we conclude that among mussels it is not possible with this technique along to identify separate genetic breeding stocks because of the extremely high proportion of adaptative plasticity that is attributable to the genotype. In fact, it is clear that where microhabitat selection is a possibility, the concept of stock will need to be very carefully defined in relation to fisheries management. It remains to determine the extent to which genotypic adaptation involves significant differences in productivity of the adult stocks. I would, therefore, like to turn to a discussion of other experiments with mussel populations which throw some light on the relative importance of genetic and environmental factors in determining population production.

The Determinants of Mussel Production

Measuring the contribution of genetic and environmental factors to production in mussels is not dependent on having identified the functions of the enzymes which we have used to signal a genetic difference. In our case, we began our production studies with the mussels before we had the evidence for such strong microhabitat effects on the genetic constitution of the stocks we were using. Earlier work (Freeman and Dickie, 1979) had suggested a strong persistence of growth rate among individuals, which appeared likely to have a genetic basis. We were interested in measuring its possible effects on a population scale.

The experimental results described here come from a triple-transfer experiment conducted over a five-year period. For the study, we chose three of the localities later involved in the isoenzyme study: Bedford Basin, St. Margaret's Bay and Ellerslie, P.E.I. In each of three successive years we collected a small sample, usually of about 200 young healthy mussels, from natural beds in each of these localities. After cleaning and holding them for a short period to ensure good condition, 25 animals of specified size-classes were withdrawn, individually numbered and suspended in a plastic cage from rafts moored at each of the three sites. Initially at each site we held 25 animals of two size-classes ($40\pm$ 2.5mm and 50 ± 2.5mm shell length) from each of the three stock sources, making a total of 150 animals at each site. The

first transfers were made in the late spring of 1976, and a second experiment was begun the same way in 1977. In 1978, 50 animals of the 40mm size class were used because we could see no differences between the size groups previously established, but in addition 50 mussels of 15±2.5mm shell length were transferred. Each sample lot was measured once a month, mortalities were noted and the cages cleaned and replaced. Observations on each lot were continued for approximately 24 months, giving us data on growth and mortality of identified individual mussels in five successive calendar years.

In Tables 2 and 3 is a summary of a 4-way classical analysis of covariance of the growth and mortality rates derived from the experiments. Both production parameters vary with time during the year and with time during the experimental holding period. After examining the data, it appeared that shell-length of the mussels was a powerful predictor of productivity, although relative growth is not linear with time. Accordingly, we adopted a measure of length and an index of weight $(1^{2.65})$ as covariates in the analysis. The growth and mortality data normalized in this fashion were then examined in the analysis of covariance. In the tables we show how the total variance is reflected in a 4-way classification of the data according to stock, site, season and calendar year, together with their 2-way interactions. In these tables we have summarized data over 3-month seasons of the calendar year, since our preliminary analysis indicated that seasonal periods of this length provided the most satisfactorily homogenous sample units for describing the amount of data we had available. The analysis provides an explanation for about 80% of the observed total variance in growth rate and 50% of the total variance in mortality.

In this analysis, the variance explained by the "stock" term is a direct measure of the relative importance of genetic constitution on the production parameters of the mussel, since each stock was reared at each site over the experimental period. By the same logic, it can be deduced that variance explained by the "site" classification gives a measure of the relative importance of what we generally refer to as "environment." In this examination of the data, the "seasonal" term is of only limited interest; we needed to examine it in detail in relation to our further study of the actual production of mussels. However, I should like to draw attention to the term

"calendar-year." In a sense, this might best be considered as a scaling factor by which one can judge the overall significance of the experimental results. That is, there is no easy a priori criterion for judging how different were the various sites chosen for our experiments in relation to the possible range of total variation in production parameters that is exhibited by the species Mytilus edulis. We have, however, some common experience of the year-to-year variations normally expected in a given environment. The fact that in these results the F ratios for site are a little more than five times the F ratios for calendar year provides some assurance that the sites chosen for the comparison are different in a significant way, relative to normal environmental vississitudes in production parameters.

Under these conditions, we have concluded that the F ratios for "stock" and "site" in Tables 2 and 3 provide us with an immediate index of the relative importance of genetics and environment on the growth and mortality of the blue mussel. What is particularly interesting to us is that the results for growth and mmortality provide a striking contrast. The Table 2 data shows that in the case of growth, "site" accounts for about three times the variance accounted for by "stock." The Table 3 data show that for mortality the reverse is the case, "stock" accounting for about twice the variance accountable to "site."

This simple interpretation is slightly complicated by the fact that in the growth data there is also a statistically significant interaction of stock and site. That is, the stocks themselves performed relatively differently in the various sites. Our conception of site and stock as primary variables is therefore somewhat naive; however, the interaction is small compared with the main effects and therefore need not seriously divert our attention from them. The interaction of stock and site is not statistically significant for mortality rate.

Conclusions from the Production Study

Growth and mortality rates comprise the most important production processes affecting a given cohort of animals, and are widely used in fisheries management calculations. However, it is common experience that growth variations are evident among population samples while mortality is usually difficult to measure. It is therefore of interest that our results for the mussel also show high variations in growth associated with environmental conditions.

The results suggest that there is a significant contribution of the genetic constitution to stock growth, and that this contribution is larger than the year-to-year variations, but it is still a relatively small component of the total variation likely to be encountered.

The mortality results are quite different. Once the strong seasonality of the mortality has been taken into account, there is apparently relatively small variation in mortality rate with time. In fact, the "calendar-year" term for mortality was statistically insignificant as an explanation of the total variance. However, the total variation in mortality among the different sample lots was very high and the results show that most of this variation is attributable to the stock term. That is, the mussel samples drawn from the three different source areas showed strikingly different patterns of mortality rates, and these differences persisted no matter where the samples were cultured. It may be of general interest to point out, further, that mortality rates were lowest among mussels taken from the Ellerslie site, which exhibits extremes in environmental variations and short production periods and would be generally considered a "tough" environment. Highest mortalities came from Bedford Basin, which has a relatively mild climate and relatively high primary productivity. We do not know whether these coincidences represent cause-effect relations, but they are in keeping with the experience of shell-fish breeders in other areas that "hardier" seed stock is found in varying environments.

The relative importance of growth and mortality as determinants of production is not readily generalizable from species to species, so our experience with the mussel does not mean very much for fish studies, and we have not shown any production calculations here. The mussel is a relatively short-lived animal and mortality rates of adults are correspondingly high. What is perhaps of more general interest is that in our experiments the variations in mortality were considerably higher than variations in growth so that any judgement of the productive capacity of either a site or a stock of mussels would be seriously in error if it were based on comparative growth studies alone.

General Conclusions

These studies of growth and mortality verify that in the mussel, genetic constitution is an important determinant of adult stock production.

It is, in fact, the most important of the factors we have so far identified. However, the parallel studies of the isoenzyme patterns of the populations show that genetic constitution of mussels populations is itself very sensitive to microhabitat variation. In fact, it is clear that the differences in genetic constitution which we found from place to place could have been caused by the effects of small environmental differences on a common breeding stock. In a sense, then, mussel data may reflect environmental differences through genotype on a relatively short time scale.

It may be that mussels represent a class of organisms which, over time, has incorporated a relatively high proportion of its total adaptative capacity into the genotype (Gause, 1947). The relation between genotypic and phenotypic variation is a rather large area of genetic study. However, in the present context, if genotypic adaptation is an especially important factor in mussels, it may be that changes in isoenzyme frequencies are better indices of differences among local groupings of this species than would be the case in fishes or other species in which the adults display versatile behavioural and physiological patterns.

Even with this limitation on comparing fish and molluscs, the demonstration that differences in the isoenzymes have indexed important differences in population productivity is of general significance. In our case, production differences apparently characterize stocks in different localities even when their separate constitutions reflect aggregation of effects in relation to microhabitat differences. The likelihood that this is generally true in the presence of genetic differences emphasizes the importance of defining breeding stock differentiation as an aspect of production studies and underlies the importance of continuing them with other species.

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TABLE 1. Heterozygote deficiency values for 5 isoenzymes at 6 localities within St. Margaret's Bay. Probability levels resulting from tests for homogeneity among loci and localities are shown in the row and column margins, respectively.

	LAP 1	PEP 2	PGM	PGI	AP	
NWC	-0.311	-0.668	-0.334	-0.577	-0.103	***
SII	-0.567	-0.526	-0.396	-0.331	-0.227	*
LI	-0.518	-0.457	-0.435	-0.350	-0.124	*
PI	-0.716	-0.304	-0.485	-0.297	-0.001	***
FP	-0.596	-0.404	-0.438	-0.302	-0.089	***
TI	-0.680	-0.568	-0.431	-0.470	-0.165	***
	**	*	N.S.	N.S.	N.S.	

TABLE 2. Analysis of covariance of seasonal growth rates.

SOURCE OF VARIATION	SUM OF SQUARES	DF	MEAN SQUARE	F	SIGNIF OF F
Covariates	9.671	2	4.835	439.539	.001
Length	2.922	1	2.922	265.583	.001
Weight	1.003	1	1.003	91.139	.001
Main Effects	4.877	11	.443	40.304	.001
Stock	.732	2	.366	33.263	.001
Site	2.136	2	1.068	97.090	.001
Season	.946	3	.315	28.655	.001
Calyear	.725	4	.181	16.471	.001
2-Way Interactions	2.032	41	.050	4.504	.001
Stock Site	.204	4	.051	4.634	.001
Stock Season	.610	6	.102	9.241	.001
Stock Calyear	.098	8	.012	1.109	.356
Site Season	.077	6	.013	1.161	.327
Site Calyear	.241	8	.030	2.742	.006
Season Calyear	.716	9	.080	7.230	.001
Explained	16.579	54	.307	27.909	.001
Residual	3.663	333	.011		
Total	20.243	387	.052		

TABLE 3. Analysis of covariance of seasonal mortality rates.

SOURCE OF VARIATION	SUM OF SQUARES	DF	MEAN SQUARE	F	SIGNIF. OF F
Covariates	10.217	2	5.108	4.414	.013
Length	9.934	1	9.934	8.583	.004
Weight	10.115	1	10.115	8.741	.003
Main Effects	116.223	9	12.914	11.158	.001
Stock	30.997	2	15.499	13.392	.001
Site	17.845	2	8.922	7.710	.001
Season	72.404	3	24.135	20.854	.001
Calyear	2.112	2	1.056	.912	.403
2-Way Interactions	152.334	29	5.253	4.539	.001
Stock Site	7.297	4	1.824	1.576	.181
Stock Season	48.843	6	8.141	7.034	.001
Stock Calyear	8.903	4	2.226	1.923	.107
Site Season	70.142	6	11.690	10.101	.001
Site Calyear	13.783	4	3.446	2.977	.020
Season Calyear	12.280	5	2.456	2.122	.064
Explained	278.775	40	6.969	6.022	.001
Residual	276.596	239	1.157		
Total	555.370	279	1.991		

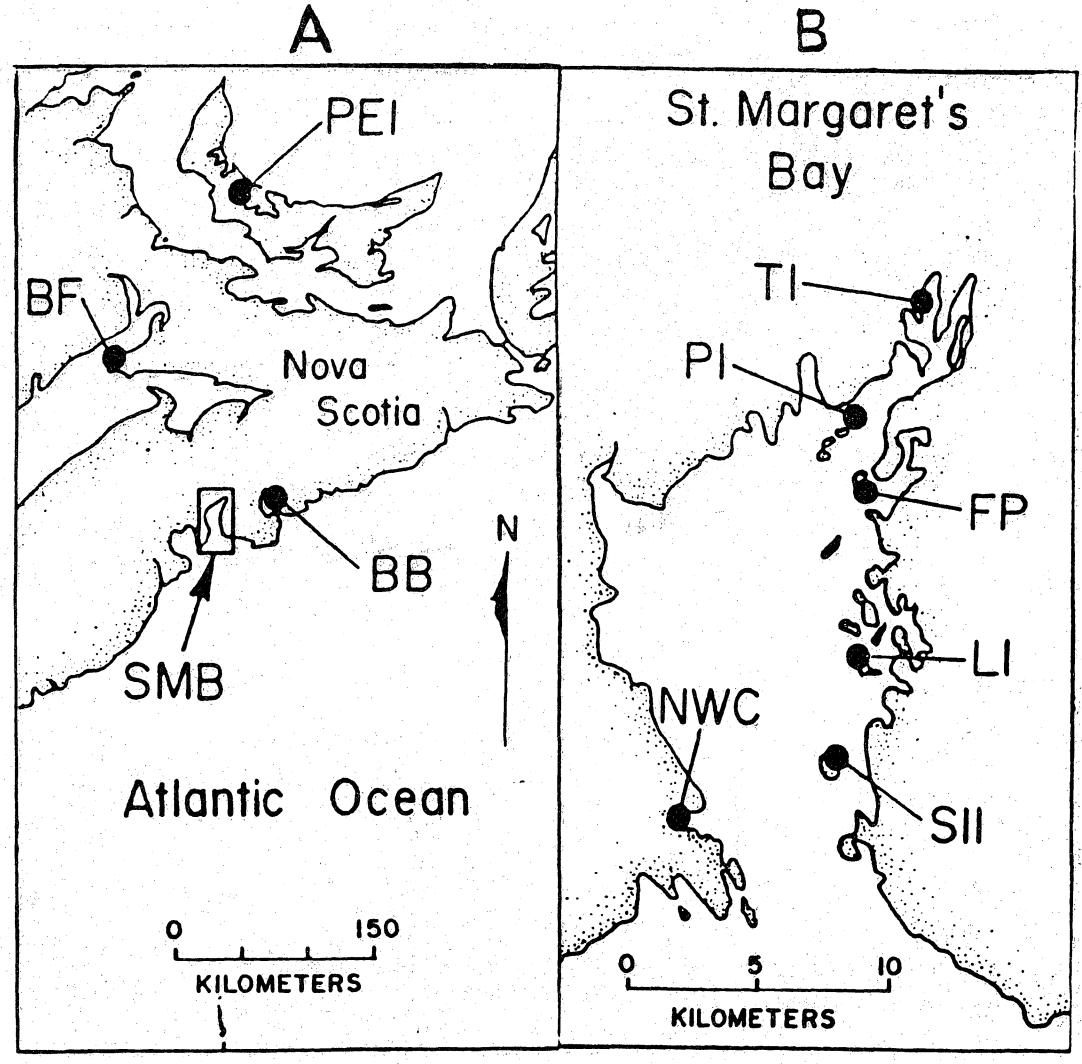

FIG. 1. Sites of collections for the (A) macrogeographic and (B) microgeographic parts of the study

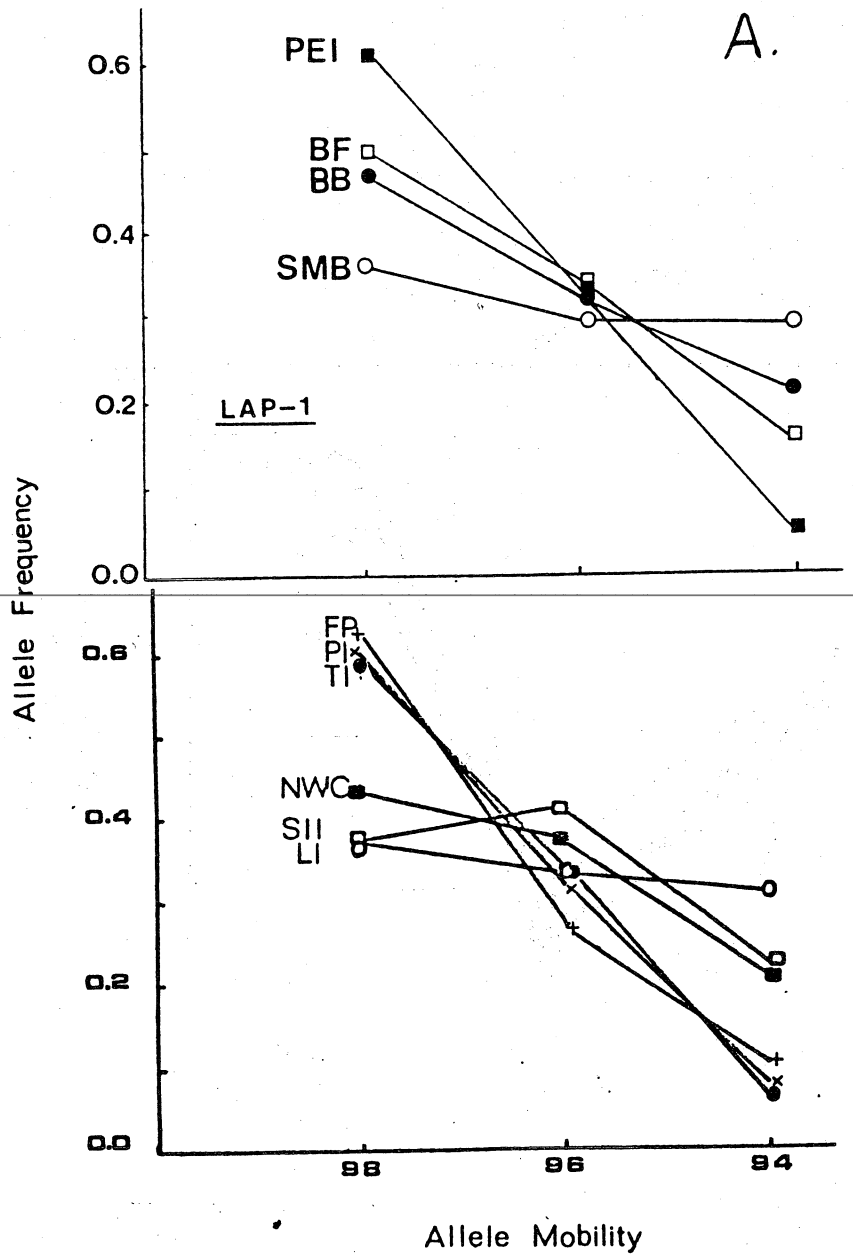

FIG. 2A. Allele frequency distributions for LAP-1.

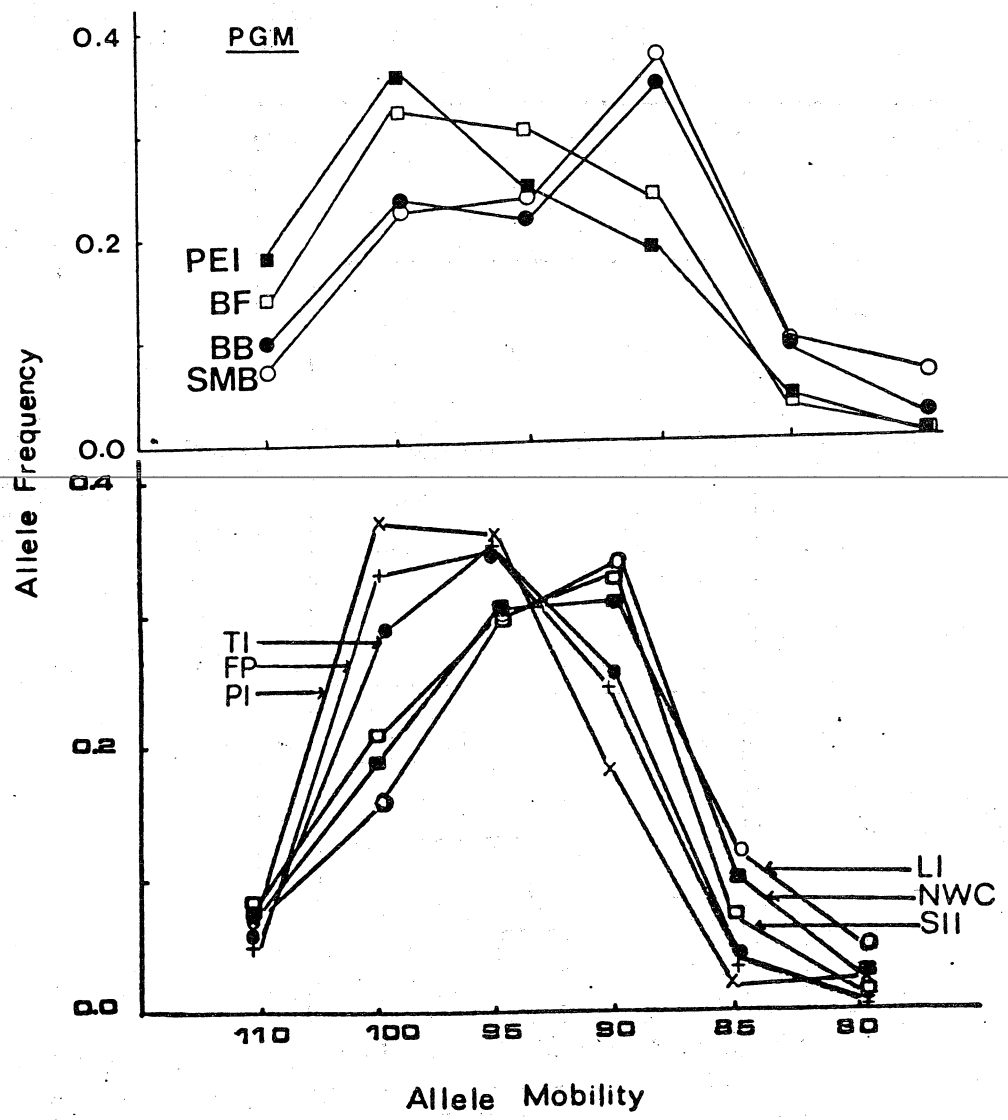

FIG. 2B. Allele frequency distributions for PGM.

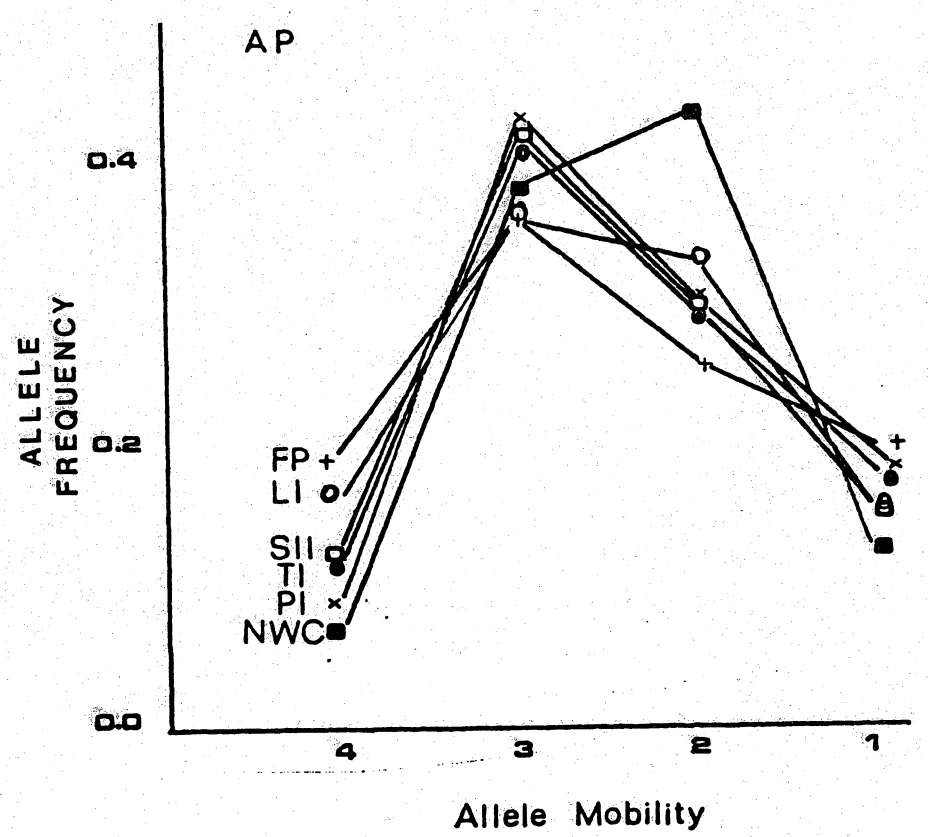

FIG. 3. Allele frequency distribution for AP.

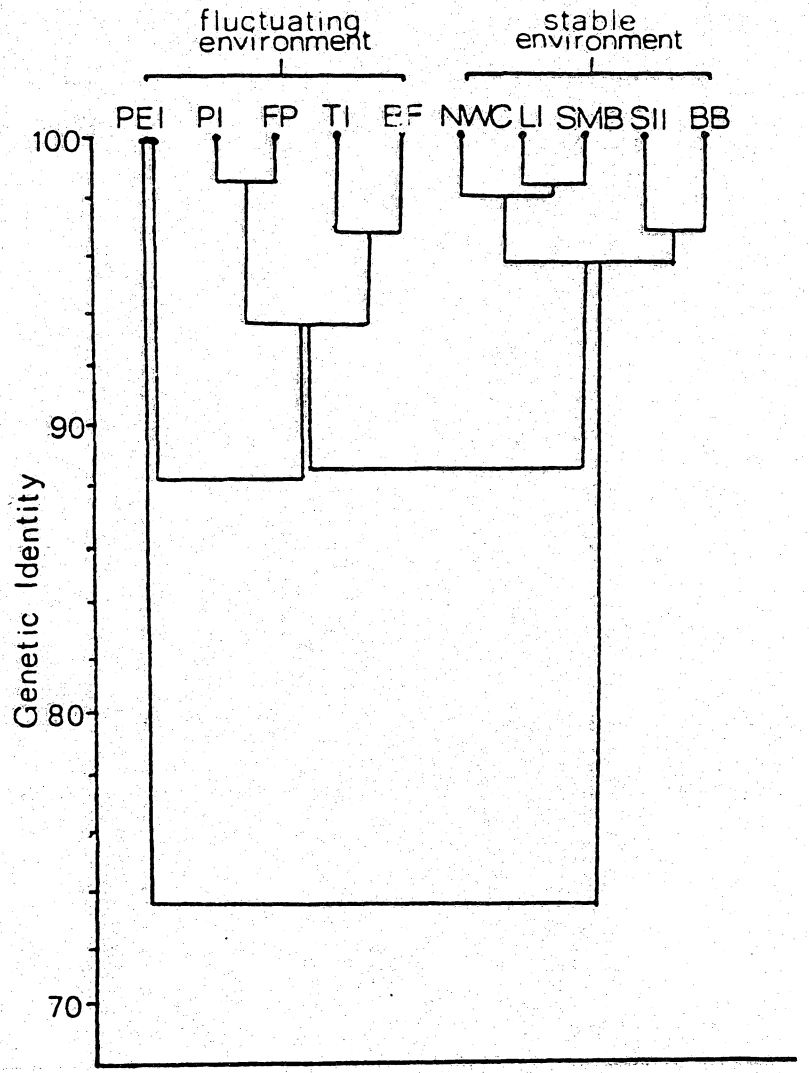

FIG. 4. A dendrogram of genetic identities based on the frequency distributions of 4 isoenzymes collected from the sites identified in Figure 1.